Biomechanics of Articular Cartilage and Determination of Material Properties

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ABSTRACT

LU, X. L., and V. C. MOW. Biomechanics of Articular Cartilage and Determination of Material Properties. *Med. Sci. Sports Exerc.*, Vol. 40, No. 2, pp. 193–199, 2008. Descriptions of the mechanical behaviors of articular cartilage and their correlations with collagen, proteoglycan, water, and ions are summarized, with particular emphasis on understanding the osmotic effect inside the tissue. First, a descriptive explanation is presented of the biphasic theory required to understand how interstitial water contributes toward the viscoelastic behavior of any hydrated soft tissue. Then, the famous osmotic effect in charged, hydrated soft tissue is interpreted in light of the triphasic mixture theory framework. In the introduction of mechanical testing methods, our emphasis is on the popular indentation technique, which can determine the material properties of cartilage *in situ* or *in vivo*. The widely accepted indentation analysis solutions in cartilage biomechanics history are summarized and evaluated. At the end of this paper, a new *generalized correspondence principle* between charged, hydrated soft tissue and linear, isotropic, elastic material (i.e., elasticity theory) is introduced. This principle makes the employment of triphasic theory as straightforward as using an elasticity theory to solve any equilibrium problem where the elasticity theory can be used to model the material. By using this generalized correspondence principle, the fixed charge density of bovine cartilage has been simply and conveniently calculated from the indentation testing data. The results of proteoglycan content from this mechanical test are remarkably consistent with those from standard biochemical assay. This new correspondence principle significantly improves the power of indentation tests in the determination of mechanoelectrochemical properties of articular cartilage. **Key Words:** BIPHASIC THEORY, TRIPHASIC THEORY, FIXED CHARGE DENSITY, INDENTATION, CORRESPONDENCE PRINCIPLE

Articular cartilage is a layer of low-friction, load-bearing soft tissue that overlies the articulating bony ends in diarthrodial joints. It provides the joint with essential biomechanical functions, such as wear resistance, load bearing, and shock absorption for eight decades or more (27). From a biomechanical standpoint, this important functional characteristic relies on the multiphasic nature of articular cartilage (21,23,28,29,34,35). In engineering terms, the tissue is a porous, viscoelastic material consisting of three principal phases (a phase represents all of the chemical compositions with similar physical properties): 1) a solid phase (Fig. 1), which is composed predominately of a densely woven, strong, collagen (mainly type II) fibrillar network (15–22% by wet weight) enmeshed with proteoglycan macromolecules (PG, 4–7% by wet weight, molecular weight of approximately 200 million daltons); 2) a fluid phase, which is water (normally < 80% by wet weight); and 3) an ion phase, which has many ionic species of dissolved electrolytes with positive and negative charges (Na^+, Ca^{++}, Cl^-, etc; << 1% by wet weight) (11,21,29,30). These three phases act together to generate the tissue that is quite remarkable in its ability to withstand enormous compressive loads (many times body weight) applied onto the tissue (40), and the associated high compressive and shear stresses. It has been reported that the compressive stresses are as high as 20 MPa in the hip, which is approximately 3000 lb per square inch (14). The ability of articular cartilage to withstand such high compressive loading without being crushed is attributable to the multiphasic nature of the tissue, and the unique combination of the related material properties of the tissue (21,31,35).

Articular cartilage is vital for maintaining the joint motion, but, when damaged, it is significantly involved in degenerative disease of joints such as osteoarthritis (OA) (29,34). OA can cause joint deformities, pain, reduced joint motion, and even absolute loss of joint function. However, joint loading and motion are required to maintain normal activities of daily living, and to maintain adult cartilage composition, structure, and mechanical properties. Reduced joint loading, in the form of immobilization or casting, incurs decreased matrix synthesis, which often leads to cartilage thinning (18), whereas the opposite weight-bearing limb often sees hypertrophy, that is, an increased cartilage matrix synthesis and content (16). Furthermore, the use of

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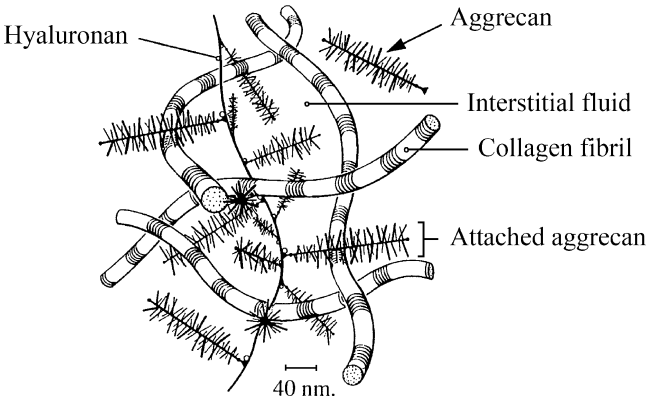

FIGURE 1—A schematic representation of the collagen network interacting with the proteoglycan network, forming a porous, fiber-reinforced, composite solid matrix in articular cartilage. The interstices of this porous solid matrix are filled with water and dissolved ions. The "average" pore size for interstitial fluid flow is approximate 60 Å.

external fixation on diarthrodial joint can produce severe degenerative lesions and chondrocyte death in the area of contact, whereas fibrillation, decreased PG content, and altered PG molecular conformation are often observed in the noncontact articular tissue. All of those biochemical and biomechanical changes attributable to immobilization are, at least in part, reversible on remobilization of the joint (41). Moreover, the effect of loading depends on loading magnitude, rates of loading (e.g., impact), and frequency. Static compression of the tissue to physiological strain magnitudes leads to the breakdown of cartilage PG (22). Moderate exercise, however, increases cartilage matrix synthesis and content and has a protective effect on the joint (19,37). This conclusion is also supported by *in vitro* experiments showing that loading with moderate frequency and magnitude increases matrix synthesis and biomechanical properties (39).

On a molecular level, the function loss of OA tissue results from the disrupted macromolecular matrix network. For example, the PG size decreases with age and fluctuate with OA stages (12,29,36). Decrease in PG content leads to the mechanical weakening of cartilage. As more PGs are depleted from the cartilage, cartilage loses its normal load supporting mechanism, and becomes susceptible to microdamage from mechanical loading, which further disrupts matrix structural integrity (3,4). Another consequence of the loss of PGs from the tissue is the loss of the fixed charges (see below) that is required to maintain overall tissue hydration and the osmotic environment and thus the ability to retain water is impaired (21,30). These are followed by an increasing rate and accumulation of microdamage to the collagen network, leading eventually to the disintegration of the extracellular matrix. From the bioengineering perspective, a first step to understand this disease process is to characterize the structure-material property–function relationships existing for articular cartilage. Specifically, it is necessary to (a) determine the mechanical properties of normal cartilage, (b) measure the biochemical composition of cartilage, especially the PG and water contents, and (c) study the correlation between its biochemical compositions and mechanical properties. These studies are essential and are absolutely fundamental for understanding the etiology of OA disease through mechanical and biochemical pathways and for furthering the search of effective techniques to avoid or retard the cascade of degenerative process *in vivo*.

This paper is aimed at introducing the reader (especially colleagues from the sports medicine sciences interested in articular cartilage research) to some recent advances and important concepts regarding the biomechanics of articular cartilage. It will address the following topics: 1) how cartilage deforms and supports load; 2) how the osmotic pressures are developed in the solid matrix ; 3) how to determine mechanical properties *in situ* or *in vivo*; and 4) how to determine the PG content using the mechanical indentation test.

BIOMECHANICAL PRINCIPLES OF ARTICULAR CARTILAGE

To know how any piece of material might function within a structure, it is essential to know the mechanical (i.e., stress–strain) behavior of the material. The relationships between the stresses and strains are known as constitutive laws. The simplest constitutive law is the linear elasticity law where the proportionality constant between stress and strain is known as the modulus (i.e., Young's modulus, Poisson's ratio). Poisson's ratio is the ratio between lateral (transverse) strain and axial strain in an axially loaded specimen. A ratio of 0.5 indicates an incompressible material, whereas a Poisson ratio of 0 indicates a highly compressible material. However, for most orthopedic tissues (e.g., cartilage, ligament, tendon, and meniscus), which contain large amounts of water, the stress–strain behavior seems nonlinear. Under compressive loading, the fluid component within the tissue may flow out of the tissue, like water being squeezed out of a sponge. The ease with which fluid may flow through a porous solid matrix is a measure of its permeability. The lower permeability (usually associated with smaller the pores), the harder it is to force fluid through the solid matrix; thus, the slower it takes the tissue to reach final equilibrium state when no motion is in progress.

Fluid flow and biphasic theory. To understand cartilage deformational behavior, it is easiest to first understand how fluid can flow through it. Physically, when cartilage is compressed, a volumetric change or a pressure gradient will be created within the tissue. As a result, the interstitial fluid will begin to flow within the tissue or be extruded from the tissue. The fluid passing through the porous solid matrix generates very high frictional resistance (i.e., drag), and therefore is the primary mechanism giving rise to the frictional dissipation responsible for the viscoelastic behavior of articular cartilage in compression. To date, the most successful theory for cartilage compressive viscoelastic behaviors is the biphasic theory developed by Mow and coworkers (35). This binary mixture theory (a branch of mechanics to study the materials with more than

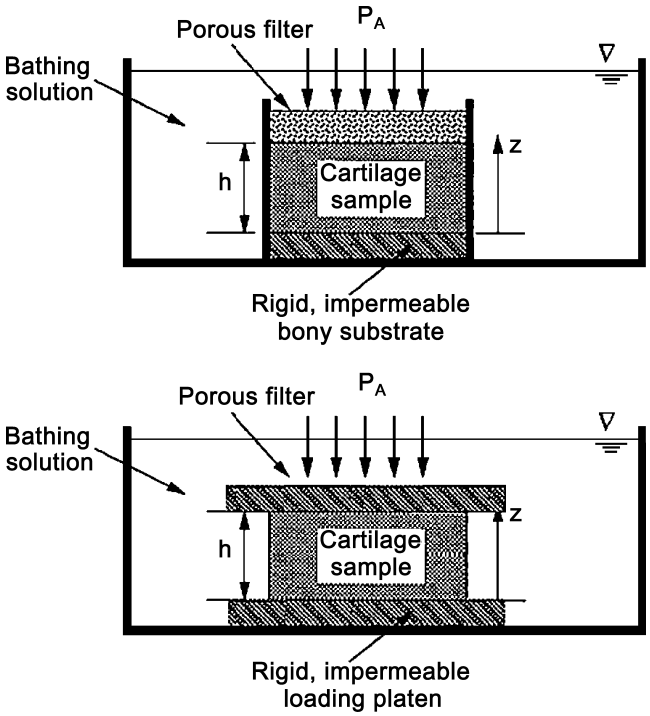

FIGURE 2—Schematic of the two configurations frequently used to study the compressive behavior of articular cartilage: confined compression (*top*) and unconfined compression (*bottom*). For the unconfined compression, the cartilage sample has to be stripped off from the subchondral bone and cut into a perfect cylinder.

one phase) models soft tissues as composite materials consisting of two phases, a solid phase and a fluid phase. The solid phase (the porous solid matrix) is elastic and permeable to fluid. According to the biphasic theory, three major internal forces act within the loaded tissue: a) the stress developed within the deformed collagen–PG solid matrix; b) the pressure that is developed within the fluid phase; and c) the frictional drag acting between the fluid phase and the solid phase as they flow past each other. In contrast, in a single-phase elastic medium, the only internal force comes from the elastic solid matrix phase (i.e., the (a) component in above described biphasic theory). In a biphasic medium, however, all of the three internal forces act in concert to balance the externally applied force, thus giving rise to a viscoelastic effect. Most importantly, the permeability of normal cartilage is extremely small ($\sim 10^{-16}$ $m^4 \cdot N^{-1} \cdot s$), indicating that immense interstitial fluid pressures and dissipations are occurring inside the tissue during compression. These mechanisms provide an efficient method to shield the collagen–PG solid matrix and embedded chondrocytes from high stresses and strains associated with joint loading during daily activities, because the pressurized fluid component provides for the major portion of load-bearing function in cartilage (43). For example, under a confined compression test of articular cartilage, it was shown that the interstitial fluid immediately pressurizes upon loading and constitutes more than 95% of the total load support (43). For normal articular cartilage mechanical properties and permeability, this high percentage of fluid load support can last more than 500 s in a confined compression test and likely much longer *in vivo*.

In the linear biphasic theory of cartilage, the simplest version of the biphasic theory, the stress–strain law for the solid matrix is assumed to be isotropic and linearly elastic, and the frictional drag acting on the solid phase can be given by an equivalent linear form of Darcy's law (35). Darcy's law correlates the fluid flow rate linearly with the tissue permeability, tissue thickness and pressure drop. Therefore only three material properties are necessary to characterize the linear biphasic material, Young's modulus E, Poisson's ratio v, and hydraulic permeability k. The Young's modulus of the solid matrix can be easily determined from an unconfined compression test (Fig. 2) (2,35). The Poisson ratio can be obtained by using an optical method to measure the equilibrium lateral expansion under unconfined compression (17). In cartilage biomechanics, instead of Young's modulus, the aggregate modulus is often used to describe the tissue, because it can be directly calculated from the equilibrium data in an confined compression test (i.e., the loading pressure divided by the equilibrium strain in compression direction) (Figs. 2 and 3). In a stress-relaxation or creep test (Fig. 3) of confined or unconfined compression, the hydraulic permeability k can be determined by curve-fitting the creep or relaxation curve generated in the test (2,35).

Charged nature of solid matrix and the triphasic theory. A major compositional factor, one that has received intense biochemical and physiochemical scrutiny during the past several decades, are the charged sulfate (SO_3^-) and carboxyl (COO^-) groups attached to the chondroitin and keratin sulfate chains that comprise the glycosaminoglycans (GAG) in cartilage (Fig. 4) (21,30,34). These negative charges give rise to a high charge density within the tissue, which is commonly known in the literature as the *fixed charge density*, or simply FCD ($mEq \cdot mL^{-1}$; ranges from 0.04 to 0.2 $mEq \cdot mL^{-1}$ in normal cartilage (29)). Because each fixed negative charge requires a mobile counter-ion (e.g., Na^+) to maintain electroneutrality within the interstitium (7,29), FCD gives rise to an imbalance of mobile ions between interstitium and the external bathing solution. This excess of mobile ions yields a pressure difference (21,31) between the internal and external aqueous solutions within and surrounding the tissue. This pressure difference generates a higher fluid pressure inside the soft tissue, which is widely known as the Donnan osmotic pressure (7,21,30). This osmotic effect can profoundly affect tissue hydration, control of fluid content, ion transport through the interstitium, and a broad spectrum of other observed mechanical responses, such as the measured apparent material properties (9,15,21).

To account for the presence of FCD and the observed Donnan osmotic pressure effects, ion transport through the tissue, and other electrokinetic effects, Lai and coworkers (21) developed the triphasic theory (a tertiary mixture theory) for

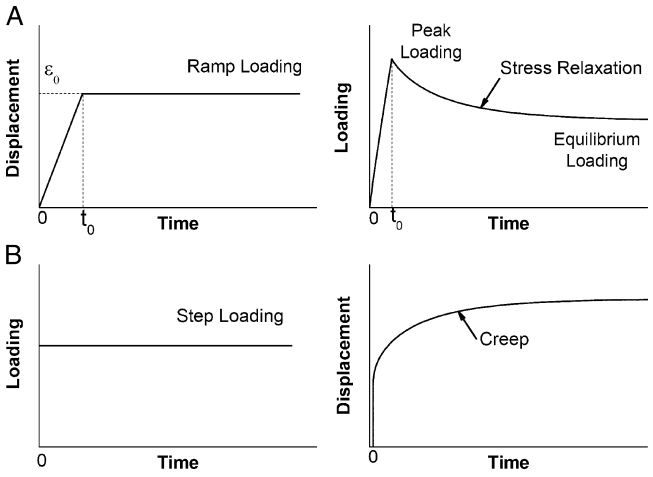

FIGURE 3—Schematics of load-deformation viscoelastic behaviors of articular cartilage. *A*, In a stress-relaxation test, a displacement is applied on the tissue at a constant rate until a desired level of compression is reached. This displacement results in a force rise followed by a period of stress relaxation until an equilibrium force value is reached. *B*, In a creep test, a step force is suddenly applied (stepwise) onto cartilage results in a transient increase in deformation (i.e., creep). In articular cartilage, the creep and stress-relaxation behaviors are mainly governed by the frictional forces generated as the interstitial fluid flows through the porous solid matrix and by the frictional interactions between the matrix macromolecules such as proteoglycan and collagen.

cartilage in 1991 on the basis of the previously described biphasic model. According to biphasic and triphasic theories, the equilibrium modulus of cartilage actually includes contributions from two sources: the Donnan osmotic pressure and the "intrinsic stiffness" of the solid matrix without charge effect (i.e., the FCD-independent component). Recent studies have shown that the Donnan osmotic pressure can contribute 30–50% of the equilibrium stiffness of cartilage tissue (~ 0.2 MPa) (8,32). To consider this effect, the concept of intrinsic and apparent properties was recently introduced in the literature (21,25). The apparent and intrinsic properties of the tissue are defined as the properties of the tissue with and without the osmotic pressure effects—that is, the properties in triphasic framework as intrinsic properties and those in biphasic model as apparent properties.

A generalized correspondence principle. The mathematics of triphasic analysis are very complex and beyond the scope of this short review. Here, we introduce our new generalized correspondence principle, recently developed by Lu et al. (24), by which the mathematical analyses required by the triphasic model can be greatly simplified. According to this principle, the equilibrium deformational behavior of a charged, hydrated tissue under loading is identical to that of an elastic medium without charge. The mechanical properties of this equivalent material can be correlated with the intrinsic elastic moduli, FCD, and free-ion concentration within the cartilage tissue by two simple equations (24). More importantly, the validity of these equations is independent of the deformation state of the solid matrix. Therefore, they can be employed for loading conditions involving arbitrary deformation fields, such as confined or unconfined compression and indentation tests, etc. This makes the employment of triphasic analysis as straightforward as using an elastic model to solve equilibrium problems, while being able to properly account for effects attributable to the FCD and the osmotic effect.

DETERMINATION OF *IN SITU* MECHANICAL PROPERTIES AND FCD

Indentation test and *in situ* mechanical properties. The indentation experiment is the most frequently used method worldwide for studying the biomechanical properties

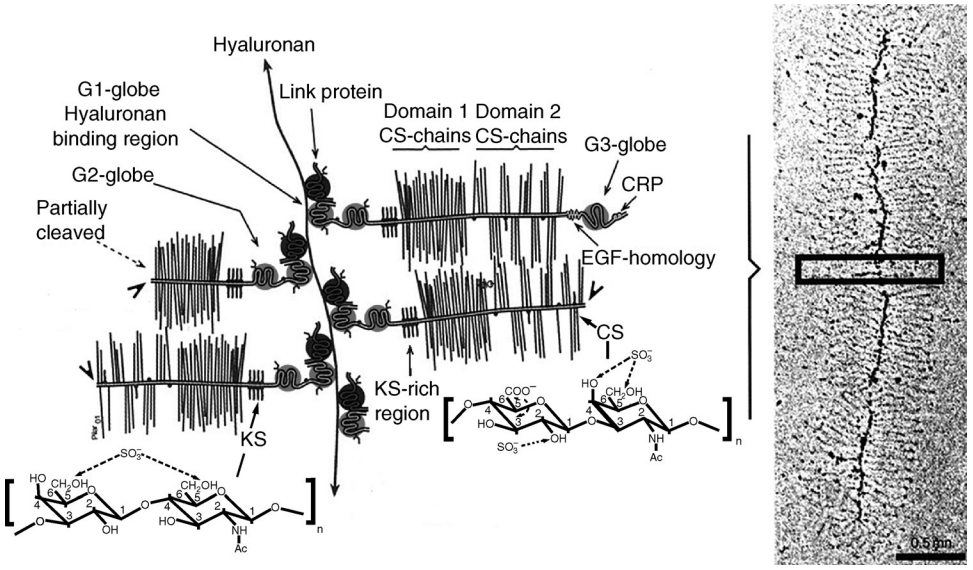

FIGURE 4—Schematic depiction of an aggrecan and its location on a PG aggregate. An aggrecan is composed of glycosaminoglycan chains (Keratan sulfate and chondroitin sulfate) bound covalently to a core protein molecule. An electron micrograph of the macromolecule after rotary shadowing is shown at right.

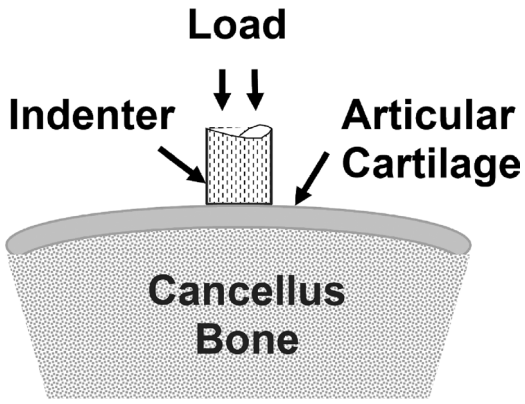

FIGURE 5—A schematic diagram of indentation test showing a cartilage bone block, indented by a circular, rigid, porous–permeable, and frictionless indenter tip.

of articular cartilage (1,13,33,42). A schematic diagram of an articular cartilage indentation experiment is shown in Figure 5. This experiment is attractive because it does not require special specimen preparation such as microtoming precise thin strips required for the tensile tests or preparing perfect cylindrical plugs for the confined compression or shear tests. Further, the indentation test has the added advantage that the material properties of cartilage are determined *in situ* on the bone, a condition more closely resembling the physiological situation. Thus, the mechanical properties obtained from indentation can provide a more accurate description and understanding of the tissue's characteristics. In addition, the nondestructive nature of indenation increases its potential to be used in clinical applications (1,20). Recently, indentation also has been popular in studies involving small joints, such as the temporomandibular joint (44), or in small-animal models, where the cartilage tissue is too thin to be harvested for other mechanical tests or biochemical assays (5,38).

In light of a biphasic solution for indentation (26), Mow et al. (33) have developed a biphasic curve-fitting strategy that can uniquely determine all three material parameters of a homogeneous, isotropic, linearly elastic matrix (Young's modulus, Poisson's ratio, and the permeability) using a single indentation test, without having to assume, out of necessity, a value for its Poisson ratio. The availability of this biphasic method dramatically improved the use and accuracy of indentation tests in the evaluation of cartilage mechanical properties.

Calculation of FCD. According to correspondence principle, with the knowledge of both intrinsic and apparent mechanical properties, the FCD can be easily calculated (24). A commonly employed method to obtain the intrinsic properties of cartilage is to perform a mechanical test by submerging the tissue in a hypertonic environment (with a high external solution concentration, such as 2.0 M) (6,8). In a hypertonic solution (2.0 M), the Donnan osmotic pressure induced by the FCD is close to zero (as can be theoretically shown (24)); thus, it is assumed that the osmotic effect from fixed charges on the mechanical behaviors is negligible (8,21). Thus, the intrinsic mechanical properties can be extracted by curve fitting the creep data obtained with the specimen in 2.0 M solution, using the indentation program developed by Mow et al. (33). In contrast, when cartilage is equilibrated in physiological condition (0.15 M solution), the mechanical loading is supported by *both* the solid matrix mechanical stress and the osmotic pressure in the fluid phase. As stated previously, the apparent properties with osmotic effect can be extracted out by curve-fitting the indentation data obtained in 0.15 M solution. With the knowledge of both intrinsic and apparent mechanical properties, the FCD can be calculated directly from the correspondence principle (24). Figure 6 shows the comparison of the FCD calculated according to this strategy and those from independent biochemical GAG assay (24,25). Linear regression analysis shows that the results from the correspondence principle and biochemical tests are remarkably consistent with each other.

In many cartilage-related studies, such as tissue engineering, evaluation of the mechanical and biochemical properties of the cartilage-like constructs are often required. The apparent mechanical properties are obtained by mechanical testing and analysis using biphasic theories. The GAG content is determined separately by biochemical assay. With the known value of apparent properties and the FCD value, the correspondence principle can conveniently determine the intrinsic properties, which is the true mechanical property of the solid matrix without the effect from osmotic pressure. In tissue-engineering studies, this could be considered as a way to determine the properties of the scaffold commonly used to grow a tissue-engineered construct. This new method can provide more accurate evaluations and insights into the features of the tissue-engineered cartilage constructs.

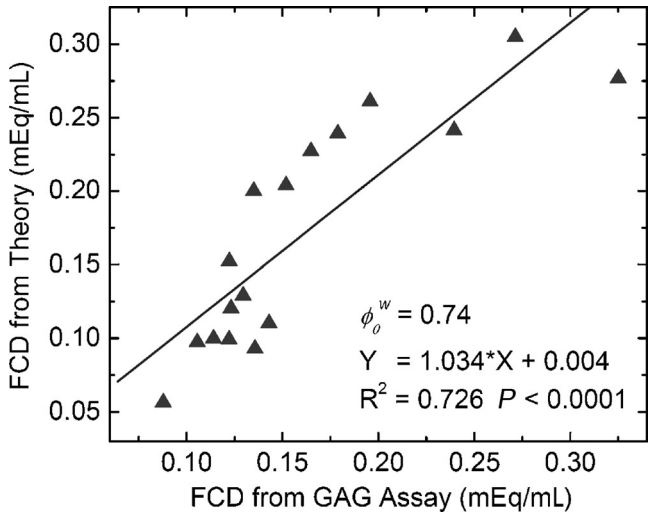

FIGURE 6—Comparison of the calculated FCD values from the correspondence principle and those obtained from biochemical GAG assay using a commercial kit (Accurate Chemical & Scientific Corp., Westbury, NY).

SUMMARY

Within the past decade, significant advances have been made in experimental, theoretical, and biological studies of the basic sciences relating to articular cartilage. New testing techniques have emerged such that articular cartilage can be studied in greater detail in terms of its nonlinear and viscoelastic behaviors, depth-dependent inhomogeneity of the mechanoelectrochemical properties, and anisotropy characteristics. New, advanced theories have been developed that encompass these experimental findings. The knowledge obtained from both fronts, experimental and theoretical, provides enrichment and in-depth understanding of the structure–function relationship in articular cartilage and in all soft, hydrated, charged connective tissues as well. There is now no doubt that each phase (the charged solid matrix, water and ions) of the cartilage contributes to its compressive, tensile, electrokinetic, and transport behaviors. The triphasic mixture theory has been successfully used to describe the flow-dependent and flow-independent viscoelastic behaviors, swelling behaviors, and electrokinetic behaviors of charged, hydrated soft tissues in the last two decades. It is widely considered in the biomechanics literature as the unified theory for such materials (10). The generalized correspondence principle presented at the end of this review paper bridges the powerful yet complex triphasic mixture theory with the simple linear, isotropic, elastic model. The simultaneously measured mechanical properties and biochemical composition will inevitably provide significant new information toward the understanding of OA etiology, especially during the early stages of the disease process.

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