Biochemical Characteristics and Water Exchange in the Surface Layer of Human Joint Cartilage

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We compared the contents of collagen, glycosaminoglycans, and various forms of water in the surface layer and whole tissue of joint cartilages of different localization. It was found that the surface layer is characterized by reduced content of glycosaminoglycans compared to the whole tissue and higher water-holding capacity due high content of bound water.

Key Words: *cartilage; bound and free water; glycosaminoglycans; collagen*

The surface layer of joint cartilages (JC) contacting with the synovial fluid is covered by a cell-free lamina (4-7 μ) and serves as a protector, which consists of intermediate and basal zones [7,10]. The surface layer matrix is composed of densely packed collagen fibers lying parallel to the cartilage surface [6,9]. Interfibrillar spaces in the surface layer are filled with a matrix consisting of water and glycoproteins [7]. Under the influence of contact pressure forces, extracellular water containing electrolytes and low-molecular-weight proteins is pressed out from the cartilage into the joint gap. After removal of the load, water returns into the cartilage and recovers hyperhydration of the cartilage [13]. Water pressed out from the cartilage assumes compression load and lubricates the joint surface. The development of osteoarthritis is accompanied by structural changes in the surface layer, its thinning and loosening [2]. The amount of bound water in JC decreases by one order of magnitude [5].

In the surface layer of JC water plays an important physiological role. However, little attention was given to the amount of various forms of water and content of biochemical components in the surface layer of JC.

Here we compared water exchange and biochemical characteristics in the surface layer and whole tissue of human JC.

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MATERIALS AND METHODS

Samples of JC of different localization (head of the femur and condyle) were obtained during postmortem examination of people (45-50 years) died from trauma. Autopsy was performed within 24 h after death. Light microscopy showed that the thickness of cartilage samples taken from the head of the femur was 200 μ. Hydration of the cartilage tissue was assayed by aquametric methods [3]. Total water content in samples was measured by electrometric titration with Fischer's reagent. The amount of freezable free water was determined calorimetrically. The amount of bound water was calculated as the difference between the contents of total and free water. The amount of water sorbed by tissues at various values of the aqueous tension (α_{max}) was estimated by the sorption-desorption method. The monolayer capacity (α_m) was calculated using Brunauer—Emmett—Teller equation [4].

The contents of hydroxyproline [15], hexosamines [11], and uronic acids [12] in the cartilage tissue were measured.

RESULTS

Collagen contents in the surface cartilage layer of the head of the femur and condyle were 8 and 16% higher than in the whole cartilage tissue, respectively (Table 1). The concentrations of hexosamines and uronic acids

TABLE 1. Content of Main Biochemical Components in the Dry Tissue of JC in the Head of the Femur and Condyle (%, *M*±7*m*, *n*=7)

Parameter	Head of the femur		Condyle	
	whole tissue	surface layer	whole tissue	surface layer
Collagen	51.48±4.2	59.21±1.8*	55.21±1.4	71.69±0.5*
Hexosamines	5.92±0.4	3.23±0.2*	5.07±0.3	2.32±0.1*
Collagen/hexosamines	8.6	20	11	30
Uronic acids	4.27±0.2	2.68±0.3*	4.22±0.4	2.48±0.3*
Collagen/uronic acids	13	22	13	28

Note. Here and in Table 2: *$p \leq 0.05$ compared to the whole tissue.

TABLE 2. Content of Various Forms of Water in JC (%, *M*±*m*, *n*=7)

Parameter		Head of the femur		Condyle	
		whole tissue	surface layer	whole tissue	surface layer
Content of water	total	70.05±0.5	78.4±0.6*	71.1±2.3	82.0±1.5
	bound	15.01±0.2	22.8±0.3*	14.9±0.9	27.2±1.2*
	free	55.04±0.5	55.62±0.6	56.2±2.0	54.8±3.2
	free/bound	3.7	2.4	3.8	2.0
(α_m)		6.3±0.4	11.0±0.5*	10.4±0.4	15.6±0.5*
(α_{max}) at humidity of 98%		37.9±1.7	44.38±1.2	42.9±1.3	59.6±2.2*

(glycosaminoglycans, GAG) in the surface layer of these cartilages were lower than in the whole tissue by 1.6 and 2 times, respectively (Table 1). The collagen/GAG ratio in the surface layer of JC surpassed that in the whole tissue by 2-3 times. Our results are consistent with published data [14].

The surface layer of JC was more hydrated than the whole cartilage tissue (Table 2). The amount of total water in the surface layer of JC in the head of the femur and condyle were 8 and 11% higher than in the whole tissue, respectively. A greater water-holding capacity of the surface layer is associated with high content of bound water, effective capacity of the monolayer (α_m), and sorption capacity (α_{max}) of biopolymers. Differences in the free water/bound water ratio were most significant. In the surface cartilage layer of the head of the femur and condyle this ratio was 1.5 and 1.9 times lower than in the whole tissue, respectively. However, the amount of free water in the surface layer did not differ from that in the whole tissue (Table 2).

Therefore, the surface cartilages layer is characterized by a lower content of GAG (most hydrophilic components) and greater water absorption than the whole tissue. Probably, the increase in the collagen/GAG ratio leads to exposure of positively charged groups in collagen. These changes increase collagen hydration and loosening of the supramolecular structure in the carbohydrate-protein matrix complex in the surface cartilage layer [1]. Considering the influence of collagen on the water-absorbing capacity of the surface layer, it should be emphasized that at maximum relative humidity practically all water in collagen is bound to active groups of biopolymers. GAG contain not only bound water, but also 2 forms of free water [8].

The matrix of the surface cartilage layer possesses high hydrophilicity, absorbs aqueous solutions of electrolytes and low-molecular-weight proteins, and transports them into deep layers. Thinning and loosening of the surface layer contribute to changes in water exchange in the cartilage tissue during osteoarthritis (*i.e.*, decrease in the amount of bound water).

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