Summary

Articular cartilage has a unique structure, which provides a smooth and friction-free motion in joints. The biophysical properties of cartilage largely depend on its biochemical composition. During aging, the changes that occur in cartilage composition affect its functionality and consequently biophysical properties. Since life expectancy increases, age-related complications like cartilage degeneration and osteoarthritis occur more frequently. These conditions may cause pain, disabilities, and dependency on assistance during daily activities which lead to an impaired quality of life; furthermore, they lead to increased health-care costs. It is, therefore, essential to learn about age-associated changes that occur in the tissue and their effects on the biophysical properties of the tissue. Therefore, this thesis had two main aims. The first aim was to investigate the biomechanical, diffusive, and biochemical changes in temporomandibular joint (TMJ) condylar cartilage in which we mimicked aging by inducing collagen crosslinks. The second aim was to improve our understanding of the effect of normal aging on biophysical (mechanical and diffusive) and biochemical characteristics of TMJ condylar cartilage.

In order to perform biomechanical characterization, it was required to measure the thickness of the cartilage via a fast, reliable, and non-destructive method. Therefore, in the first experimental chapter of this thesis (Chapter 2), we developed an ex vivo thickness measurement method for condylar TMJ cartilage, using micro-computed tomography (μCT). A thickness distribution map obtained with this method showed that the anterior region of porcine condylar cartilage was the thinnest region of the mandibular condyle. The values obtained with μCT coincided with those obtained with histology. A third method, needle penetration, overestimated the thickness due to the penetration of the needle to the first layer of subchondral bone of young condyles. Thus, we used the μCT method for the samples analyzed in the next experimental chapters.

In Chapter 3, we induced an aging-like effect of increased collagen crosslinks in condylar cartilage of young pig samples with different concentrations of ribose. The amount of collagen crosslinks increased with ribose incubation; 50 times more crosslinks were found with the highest concentration of ribose. A positive correlation was found between the level of collagen crosslinks and compressive stiffness similar to those previously described for hyaline cartilage of other joints. The treatment also changed the orientation and packing of collagen fibers in the superficial layer of the cartilage due to interfibrillar bonds. This resulted in packing of collagen fibers next to open areas between fibers. Thus, this model, in which ribose was used to mimic certain aspects of age-related changes, might be employed as an in vitro model to study age-related mechanical changes in the TMJ condyle.

To elucidate the effect of crosslinking effect of natural aging on the diffusion, we induced an increased number of collagen crosslinks in a young equine cartilage samples in Chapter 4. The effect of an increased amount of collagen crosslinks on electrostatic interactions or steric hindrance was analyzed using a neutral
and a negatively charged contrast agent. After ribose incubation of young equine TMJ samples, the crosslink level increased substantially; becoming even higher than what we observed in old equine samples (Chapter 5). As a consequence, the tissue stiffness increased 1.5 fold. Nevertheless, the diffusion changed neither for the neutral contrast agent nor for the negatively charged one. The results of this study strongly suggest that collagen crosslinking in TMJ condylar cartilage does not affect diffusion. However, it increases the stiffness for both equine and porcine (Chapter 3) TMJ fibrocartilage.

To investigate the effect of normal aging on TMJ condylar cartilage, we collected equine samples from animals of different ages. Biochemical and biophysical alterations of the mandibular condyle of different ages were assessed in Chapter 5. We showed that with aging, the diffusion of a neutral contrast agent decreased due to a decline in tissue water content, concomitant with an increase in collagen, crosslink, and collagen content. This confirmed the steric hindrance effect of aging-associated changes on the diffusion of neutral contrast agents. Surprisingly, diffusion of the negatively charged contrast agent was hardly affected. This could be explained by a remarkable change in the distribution of glycosaminoglycans (GAGs) in the tissue matrix upon aging. It also might explain the unexpected results of tissue stiffness: stiffness did not change with aging, while the amount of collagen crosslink increased 15 fold. GAGs appear to contribute significantly to the mechanical response of cartilage; it seems that the change in GAG distribution reduced the effect of the increased amount of collagen crosslinks.

We conclude that the contribution of factors that change due to natural aging is not necessarily similar to artificially induced aging by increasing the amount of crosslinks. So it seems that mandibular condylar cartilage also in this respect differs from hyaline cartilage. Of the latter cartilage it was shown that a positive correlation exists between collagen crosslink number and stiffness during aging. Collectively, we improved our understanding of the effect of normal aging on biophysical (mechanical and diffusive) and biochemical characteristics of TMJ condylar cartilage.