

Setting up *ex-vivo* biomechanics studies

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Ex-vivo models (body parts isolated from cadavers) are regularly used in orthopaedic clinical research to study implants behavior and test the performances of various surgical procedures.

Bone specimens: Bone mechanical properties are very much dependent on the water content of the bone; dry bone has been demonstrated to become more brittle, more fragile than hydrated bone. The commonly recommended measure is to harvest the bone in the first hours after the death of the animal and keep it frozen at -20° , wrapped in sponges soaked with saline. When freezing, the specimens should be wrapped in saline soaked sponges so that moisture content is maintained. Adding saline to the container prior to freezing increases the bending toughness (energy absorbed to failure) but has no effect in torsion. At the day of the test, the bone specimens should be slowly thawed at room temperature. During the test, they should be regularly sprayed with saline.

Joints specimens: Ex-vivo articular models are more complex than bone models alone. On top of bone tissue, ligaments, possibly tendons, and articular cartilage must also be preserved as well as, ideally, the synovial fluid. **Articular cartilage:** chondrocytes survive several days after death of the body. Composed of only 10% of cells, cartilage has only modest requirement for nutrients and oxygen. Storage to -20°C and -80°C does not alter the mechanical properties of articular cartilage when combined with a rapid thawing protocol; hence the tissue may successfully be stored at subzero temperatures. **Synovial fluid (SF):** Absence of synovial fluid during tests has likely an influence which is overlooked in most mechanical tests, when joint capsules are incised to allow intra-articular manipulations (e.g. ligament transection) and sutured afterwards. If hyaluronic acid is responsible for lubrication of soft-tissue (synovial membrane), lubricin, a glycoprotein present in the synovial fluid, is responsible for the lubrication of cartilage; protein concentration in the synovial fluid has been demonstrated to decrease with time (hours) particularly with higher storage temperature (20° versus 4°). Synovial fluid has visco-elastic properties. In slow motions, it acts mainly as a viscous material (viscosity = capacity of a fluid to resist shear forces). At faster motions, elastic properties progressively predominate (elastohydrodynamic lubrication), allowing the SF to share the loads imparted to joints with the articular cartilage and subchondral bone. Most of the tests carried out in mechanics laboratories are done at low to very low speed (fraction of 1 millimeter/sec), hence not reproducing physiological loading features. By the way, this comment applies also to other articular anatomical structures with a visco-elastic behavior (stress-strain relation being a function of the loading rate!) **Tendon:** Freezing/thawing has no influence on the tendinous relaxation, but alters significantly the ultimate tensile failure and Young's modulus of the tendons. The elastic modulus decreases from a mean value of 244 MPa to a mean value of 180 MPa after freezing. The most likely explanation for this is that freezing induce a deep dehydration of cellular and elastic structures, associated with their partial or total destruction. **Ligament:** the mechanical properties of the medial collateral ligament substance (rabbit knee), as represented by the stress-strain curves, tensile strength and ultimate strain also does not change following storage. Proper and careful storage by freezing would have little or no effect on the biomechanical properties of the ligaments.

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