STRUCTURAL ANALYSIS OF C AND CM HYDROGELS

Assay: Cryofixation – slicing – light microscopy

Hydrogel discs (6mm diameter and 0.5 mm thick) were stained with eosin (500 mg eosin in 100 ml 70% ethanol). Afterwards, the discs were briefly washed in PBS (pH 7.4) and the excess of the buffer was absorbed with filter paper. The discs were frozen on cryobar at -40^{o} and mounted in Thermo Scientific™ Shandon™ Cryomatrix™ mounting medium. The discs were sliced parallel to the surface in 8 μm sections by cryomicrotome (CryoStar NX70, Germany). The slices were mounted on microscope glass slides (Superfrost Plus, Menzel-Gläser, Germany) and allowed to dry at room temperature for 15-30 min. Then the slides were briefly immersed in Leica ST Ultra solvent and covered by cover medium (Leica CV Ultra) and coverslips. The slices were visualized by stereomicroscope.

Results

The C gel structure has highly expressed spatial orientation. The hydrogel consists of parallel fibers interconnected by short crosslinking fibers that make 5 - 30^{o} angles with the main structure forming ones. The main fibers lie in parallel with the hydrogel disc surface. The structure is evenly distributed moving between disc surfaces: all slices show similar structure pattern. Approximate interfiber space estimated from these images varies from 15 to 85 μm. However, this estimation as well as crosslinking fiber angle size measurements could be affected by displacing fibers apart from each other by microtome slicing procedure and should be defined more precisely by examining more samples sliced in different angles. Along main fiber direction and transverse main fiber direction are of particular importance.

The CM gel has porous structure. Pore diameter is highly variable. The hydrogel structure pattern is similar from slice to slice indicating even spatial distribution. However, this has to be confirmed by further examining the gel. At least one more microtome slicing angle perpendicular to the disc surface is strongly recommended.