Experimental report

19/08/2024

Proposal: 9-11-2124 **Council:** 4/2023

Title: Dynamic characterization of nanofibrous polymer membranes for tendon regeneration

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Elena DEL FAVERO

Experimental team: Francesca NATALI

Local contacts: Francesca NATALI

Samples: Polyhydroxybutyrate (PHB) + gelatin

Instrument	Requested days	Allocated days	From	To
IN13	10	9	18/09/2023	30/09/2023

Abstract:

Aim of the project is to get information on water permeation and local surface mobility of nanofibers-based membranes developed by means of centrifugal spinning and to be used as scaffolds to enhance the regeneration of the tendon tissue. The composition and the preparation protocol have been formulated in order to obtain membranes insoluble in aqueous fluids, suitable to support cell growth, migration and proliferation while providing mechanical support during the entire regeneration process. The scaffolds are based on polyhydroxybutyrate (PHB), a thermoplastic biodegradable and biocompatible polymer, which is gaining great interest in the medical field due to its promising properties.
We plan to compare a basic scaffold of PHB alone with membranes combined with gelatin and enriched with magnetite nanoparticles, intended to improve the environment conditions for tissue repair.

Report

For the neutron scattering experiments, rectangular dried cuts (3 × 5 cm2) of polymeric scaffolds were enclosed in air-tight aluminum flat cells of known mass, weighted and located in place for measurement. Subsequently, cuts were extracted from the aluminum cells, carefully dried and desiccated, and then fully immersed in D2O. After draining the excess D2O, scaffolds were enclosed again in the original air-tight aluminum cells, then weighted and measured. The same procedure was repeated for H2O-hydration. The hydration level h ($h = g_{water}/g_{scaffold\text{-}dry}$) for all samples was calculated to be in the range 3.2-3.3.

Elastic neutron scattering (EINS) spectra were collected at IN13 with an energy resolution of 8 μeV and an accessible momentum transfer range of $0.2 < Q < 4.9$ Å−1.
Temperature dependent scans were recorded for the different scaffolds in the dry and D2O or H2O hydrated conditions. Data were acquired in the temperature range 278–315 K, above the freezing point of the water in order to avoid coherent scattering contributions arising from ice Bragg reflections.The neutron transmission values, higher than 85%, were chosen in order to optimize the signal-to-noise ratio while keeping low enough the probability of multiple scattering events to be neglected The standard reduction of raw data (empty can subtraction, sample transmission correction, and normalization using a vanadium scan) was carried out using the LAMP Software ~~[Richard, D.; Ferrand, M.; Kearley, G. Lamp, the large array manipulation program. J. Neutron. Res. 1996, 4, 33–39].~~ . ~~Also the scattering contributions from MMT and H20 were estimated and assumed to be 11% from MMT in the CHS-MMT dry compound and 70% from H2O in both CHS and CHS-MMT.~~

In figure (left panel) we report the Mean Square Displacements (MSD) calculated from the slope, in the low-q region, of the semi-logarithmic plot of the elastic neutron scattering intensity, $S(q, \omega = 0)$, collected at different momentums transfer q for CHS, and CHS-MTT scaffolds.
The effective average force constant, <k>, calculated from the slope of MSD as a function of temperature, is shown in figure (right panel).

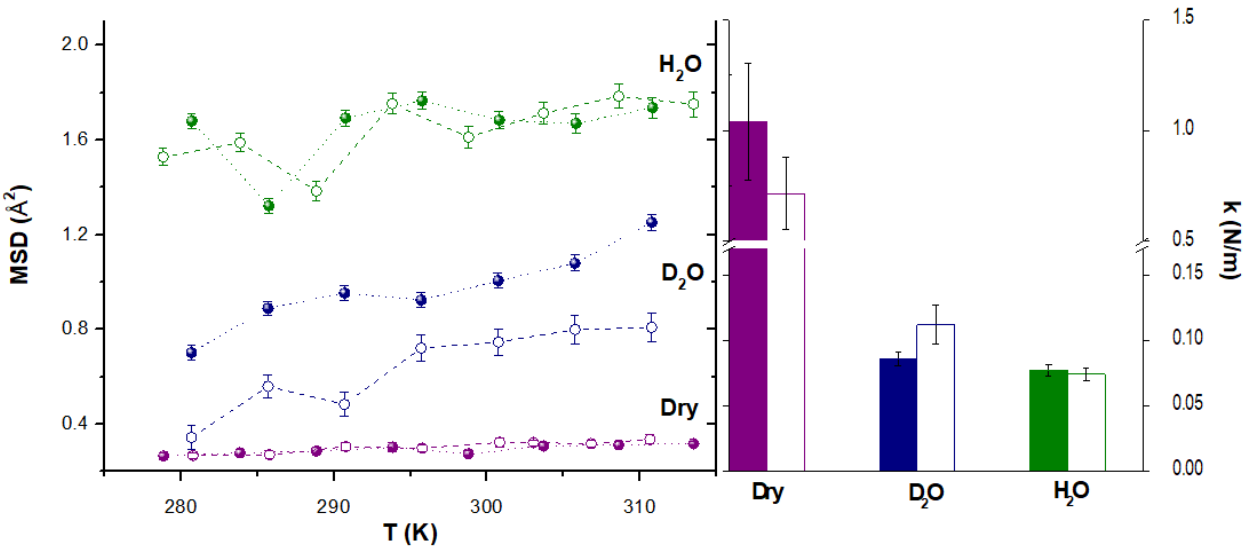

Figure MSD (left panel) and force constants (right panel) calculated for CHS (filled symbols/columns) and CHS-MMT (empty symbols/columns). Purple= dry scaffolds; Blue: samples hydrated in D2O; Green = samples hydrated in H2O.

The local dynamics in both CHS and CHS-MMT is clearly enhanced upon H2O or D2O hydration with respect to the corresponding dry states, in which the addition of MMT clay induces no significant differences. This is also reflected, as a consequence, in the k histogram, where much higher force constants are found for the dry samples (but similar, within the error bars, among them) with respect to H2O and D2O hydrated ones, indicating higher stiffness.

In the H2O hydrated samples, the high scattering contribution from water eventually hides any changes in the scaffold dynamics upon the addition of MMT (similar MSD and k values) even though, we remind that only confined, or partially-bounded, water molecules, with retarded dynamics, may be visible on IN13, given its energy resolution, in which free water contribution appears as a flat background.

A clear difference is instead observed in the D2O hydrated samples, in which the contribution of water is hidden thanks to the isotope exchange H-D. The relatively low scattering contribution from MMT with respect to the matrix (CHS), 11%, allows us to safely state that the addition of MMT clay stiffens the scaffold's local dynamics (lower MSD and higher k value).

Results show that in hydrated conditions both the amplitude of the local mobility of molecular groups within the scaffolds and their resilience are dramatically affected, resulting in a lower stiffness on the nanoscale, with MTT-doped hydrated scaffolds slightly more rigid. Cells adhering to the scaffold experience its mechanical properties at the nanoscale, which may have an effect on their growth and proliferation.