

FT-IR studies. Furthermore the swelling is higher for the squeezed or stressed hydrogel than for the native one. Upon application of a shear stress, such as the passage from a syringe needle, the strength of the interactions present in the hydrogel decreases, making the hydrogel softer. Basically, a different arrangement of water molecules from a bound state to the interstitial state occurs, as ascertained by the curve-fitting analysis of the FT-IR spectra of the swollen polysaccharide hydrogel, leading to a decrease in the mechanical properties of the hydrogels. With native-based hydrogel, the polymer-water interactions occurring via hydrogen bonds are quite strong, and are responsible for the higher values of  $G'$  and  $G''$  when compared with the same hydrogel squeezed (Pasqui et al. 2012) (Fig. 6). Table 1 highlights the storage modulus and elastic modulus of some polysaccharide hydrogels before and after the passage through the needle of a syringe. Some examples are represented by carboxymethylcellulose (CMC), hyaluronane (Hyal), alginic acid amidated (AAA), carboxymethylcellulose amidated (CMCA) and chitosan hydrogels (Barbucci et al. 2006; Fini et al. 2008; Leone et al. 2008). These properties highlight the injectable nature of these hydrogels, which is a very useful property for biomedical applications. A medical application for the osteoarthritis therapy was envisaged using polysaccharide hydrogels loaded with an anti-inflammatory drug, ibuprofen. The fact that such polysaccharide hydrogels, thanks to their thixotropic properties, can be easily injected *in situ* makes the matter easier. Animals treated with the injection of the Hyal hydrogel-Ibuprofen in the knee joints revealed a repairing mechanism relieving the pain for the patient (Barbucci et al. 2005).

The hysteresis loop is also present in another polysaccharide: Guar Gum, a non-ionic hydrophilic compound extracted from the endospermic seed of a plant (Barbucci et al. 2008). The loop demonstrates the thixotropic nature of the hydrogel. Furthermore, in order to verify visible morphological changes in the structure of the polysaccharide hydrogel and an eventual rearrangement of the structure, SEM analysis were performed before and immediately after the mechanical stress. The native structure of the Guar Gum hydrogel is compact; on the contrary after the mechanical stress the gel shows a porous structure. After four days of settling the gel regained its typical compact native structure (Fig. 7).

AFM analysis was also performed on the same hydrogel to obtain high-resolution images of small areas. The morphology of the native hydrogel is typical of a soft material with different non-homogeneous areas. The morphology of the same Guar Gum hydrogel after being subjected to a shear rate of  $700 \text{ s}^{-1}$  is completely different.

**Table 1.** Storage modulus ( $G'$ ) and elastic modulus ( $G''$ ) for 3 different polysaccharide hydrogels in the native form and after being squeezed (Camponeschi et al. 2015).

Hydrogel	State	$G'$ (Pa)		$G''$ (Pa)	
Carboxymethylcellulose	Native	550	$\pm 30$	25	$\pm 1$
	Squeezed	240	$\pm 20$	20	$\pm 2$
Hyaluronic acid	Native	970	$\pm 25$	65	$\pm 10$
	Squeezed	340	$\pm 20$	45	$\pm 5$
Chitosan	Native	4350	$\pm 650$	165	$\pm 45$
	Squeezed	3460	$\pm 150$	230	$\pm 15$