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An Artificial Disc: Chemical and Biomechanical Analysis

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Summary: Degeneration of intervertebral discs is the most common cause of back pain. The first phase of this degenerative process involves the nucleus pulposus. A rapid recovery of this structure can prevent further degradation of the annulus fibrosus. Guar Gum is an hydrophilic polysaccharide extracted from the seed endosperm of a plant. Through a cross-linking procedure a Guar hydrogel was synthesized. The Guar hydrogel could be injected by a syringe and it is a good candidate as a nucleus pulposus substitute. For the preparation of the artificial fibrosus annulus, commercially available polymer materials are tuned to achieve suitable mechanical properties. To achieve this peculiar behaviour, the main strategy investigated is the dispersion of hollow polymer micro spheres in a thermoplastic polymer matrix. Processing conditions and particle content are finely tuned to get the target mechanical behaviour.

Keywords: annulus fibrosus; guar gum; hydrogel; intervertebral disc degeneration; nucleus pulposus

Introduction

The intervertebral disc (IVD) allows motion between adjacent vertebrae while being able to resist to compressive and bending loads and, to a lesser degree, torsional and shear loads. The disc is composed of three histologically different and functionally and physically interdependent elements: the nucleus pulposus, the annulus fibrosus, and the cartilaginous endplate. The nucleus pulposus[1] (NP), is surrounded by an envelope made of a fibrous foil, the annulus fibrosus (AF).The mechanical function of the disc is so defined: the AF gives stability to the intervertebral body, enclosing the NP and holding up to tensional states, thus the NP distributes the compressive loads between the vertebrae.[2]

One of the most common causes of pain in the lumbar region of the vertebral column is the degeneration of the intervertebral disc connected to the collapse or to its herniation with consequent lost of functionality. A traumatic rupture of the annulus fibrosus may cause a herniation of the nucleus pulposus. In this situation, an adequate treatment may be the removal of the herniated nucleus and its replacement with a prosthetic device, followed by the closure of the annulus defect. Another surgical treatment is the fusion, that involves the introduction of some screws in the vertebrae, two or more, joined by a plate or a bar. In this case there will be a loss of mobility and functionality, and sometimes a degeneration of the adjacent discs. This behaviour can be explained by the mechanical stress of the region near the fused vertebrae. On the contrary the replacement of the only nucleus has several advantages. In fact, first of all, this technique is less invasive, and

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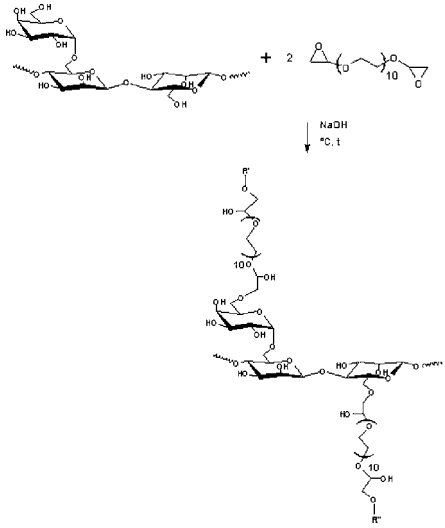
secondly, the remaining disc tissues, i.e. the annulus and the endplates, are preserved as along with their functions. Finally, the height and the mobility of the IVD are maintained and the overloading of the adjacent levels, which is often a result of spinal fusion, is prevented.

The objective of this project consists in the realization of an advanced intervertebral disc prosthesis with structural and biomechanical characteristics more similar to those of natural tissues.

Materials, Methods and Results

Guar Gum (GG; purchased by Sigma-Aldrich) was used as hydrogel for nucleus pulposus replacement mainly for its thixotropic behaviour. The crosslinking of the polysaccharides was obtained using a PEG derivate as crosslinking agent.



The hydrogel was characterised by FTIR-ATR analysis and water uptake measurements. The rheological analysis was performed to study the hydrogel behaviour under dynamic conditions, i.e. to assess and compare its viscoelastic behaviour with that of non–degenerated human nucleus pulposus.

GG hydrogel showed mechanical characteristics similar to that nucleus pulposus (Table 1).

Table 1.
Human Nucleus Pulposus (NP) mechanical characteristics compared to GG hydrogel.

	G^* ($\omega = 1$ rad/s)	G^* ($\omega = 10$ rad/s)	G^* ($\omega = 100$ rad/s)
NP	7 kPa	11 kPa	20 kPa
GG	31 kPa	33 kPa	36 kPa

The hydrogel was able to become fluid under appropriate mechanical stimulus and, once the mechanical stress was removed, it resumed its original consistence. All that means the GG hydrogel can be injected by a syringe with a very mini-invasive procedure to replace the degenerative nucleus pulposus.

Furthermore, nucleus pulposus became slightly more dissipative with frequency, whereas the GG hydrogel did not change its dissipative behaviour respect to NP.

The surface morphology of native and mechanically stressed GG hydrogel was analysed by Atomic Force Microscopy (AFM) (Solver Pro, NT MDI Instruments, Russia). AFM images were acquired in air in non-contact mode on five different areas for sample (three samples for type) with a sharpened gold coated silicon tip with a spring constant of 2.5–10 N/m and using a nominal resonance frequency between 120-180 KHz. The procedure for preparing the hydrogel for AFM analysis was similar to that reported in literature.[3] Briefly, Guar hydrogel was removed from the rheometer plate and placed on a glass coverslips, which guarantees a flat surface and then let to dry at room temperature until it was possible for the tip to approach the surface. This time was estimated to be around 30 min and strictly dependent on the amount of hydrogel. The same procedure was applied for the native guar hydrogel. The surface topography (Figure 1) was analysed after 30 min, 2 h, 3 h and 24 h on the same area of the sample to investigate the surface topography during the time.

A thixotropic material is generally made by nanometric particles that under quiescent conditions make the material stiff thanks to the numerous points of contact

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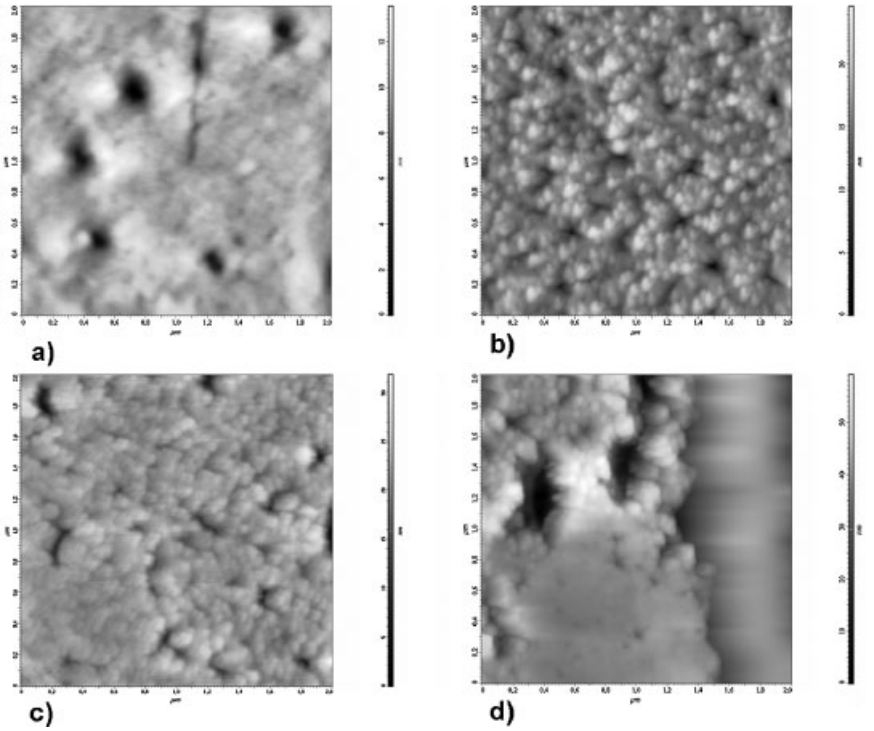

Figure 1.
AFM analysis, a) native GG hydrogel; b) after 2 h; c) after 3 h; d) after 1 day.

among the particles.[3] When a shear stress is applied, the particles begin to slide one to the others with a final collapse of the structure leading to the sol-gel transition. Once the external mechanical stress is removed, the material rearranges itself because of the nanoparticles Brownian motion, and it comes back to its original morphology.[3] The guar hydrogel appearance was typical of a soft material, as an hydrogel is, with different non-homogeneous areas. In Figure 1a is reported the morphology of the same guar hydrogel after being subjected to a shear rate of 700 s^{-1} during the Steady Flow test rheometer measurement. The surface morphology of the hydrogel at the same scan size, was completely different. It was noticed the presence of nanoparticles (average diameter of 27.4 ± 5.0 nm) all over the gel surface with no particular orientation. The same area was viewed after the hydrogel had been under quiescent condition for 2, 3 and 24 hours. It was observed that, with time increasing, the particle diameter increased (51.9 ± 9.1 nm) and the particles started to interpenetrate one into the others leading the hydrogel to its original morphology. After 24 h, nanoparticles are restricted only to some points of the surface, the other parts are completely rearranged in a homogenous structure. These observations lead to the conclusion that the GG hydrogel behaves like a thixotropic material, in other words, when it is exposed to an external mechanical stress it loses its structure and consistency, but once the mechanical stress is taken away it is able to rearrange itself coming back to its original appearance. SEM analysis (Figure 2) performed immediately after the mechanical stress showed a porous structure of the GG hydrogel with respect to the native structure of the same hydrogel which is more packed. After 4 days of rest

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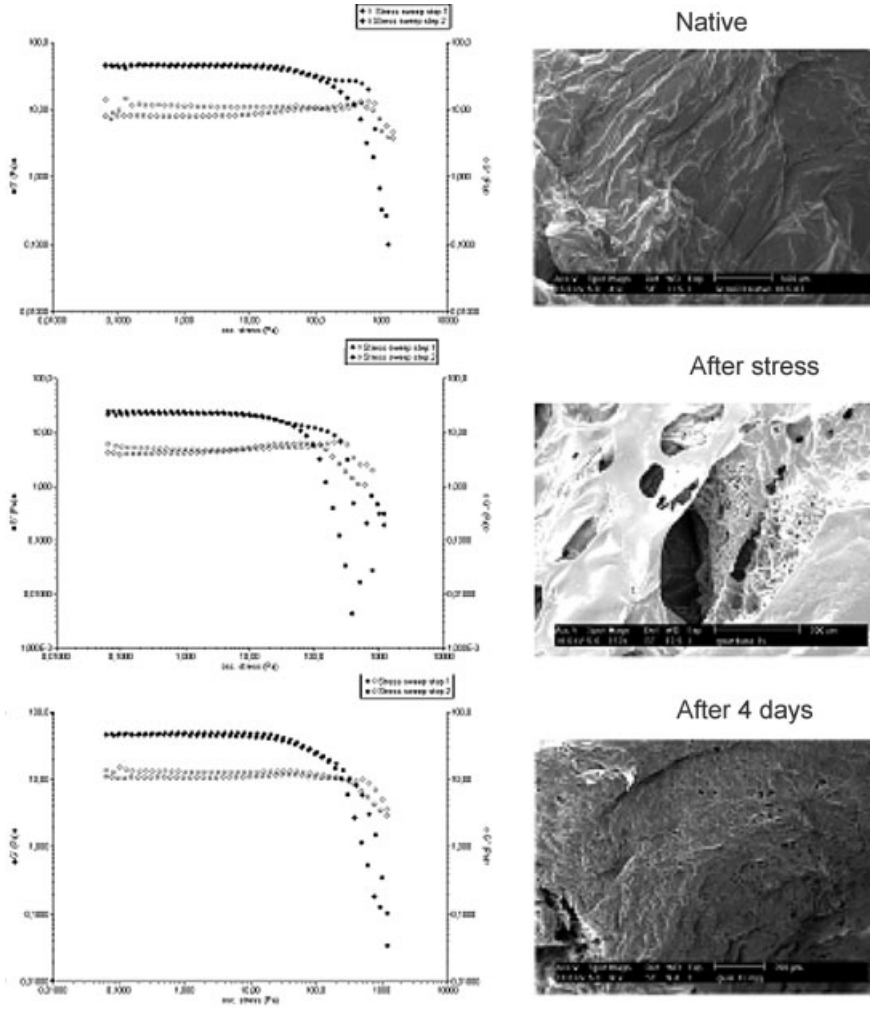

Figure 2.
SEM analysis of GG hydrogel before and after a mechanical stress.

the gel regained its typical native structures. This behaviour is confirmed by the ''Stress sweep'' tests which showed a decrease of G′ and G″ after the mechanical stress in comparison with the values of the native GG hydrogel. The G′ and G″ after 4 days of rest assumed the same values of the native hydrogel emphasizing the thixotropic nature of the GG hydrogel.

Annulus Fibrosus

Hollow microparticles (Expancel 092 DU 120, Akzo Nobel) were dispersed in low density polyethylene matrices (LDPE; Riblene FL34, Polimeri Europa) in the melt by means of a Brabender mixer at $T = 140\,^{\circ}C$, 50 rpm, $t = 8$ min. Micro particle content was set in the 0.5 and 2.0 wt % range. Thin films were prepared using a Campana PM20/200 hot melt press, at $T = 140\,^{\circ}C$, 1 min (1 atm) + 1 min (5 MPa). The mechanical behaviour of the materials, in terms of stress-strain curve, was investigated by means of a Tilnius Olsen H10Kt, equipped with a 500 N load cell, at room temperature and at a crosshead speed of

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Table 2.
Mechanical properties of PE-based formulations containing hollow microspheres.

Entry	Microspheres		E-Mod	Stress at Yield	Elongation at Yield	Stress at Break	Elongation at Break
	mg	wt %	(MPa)	(MPa)	(%)	(MPa)	(%)
PE_140	0	0	294 ± 21	9.7 ± 0.5	9.1 ± 0.7	12 ± 1	231 ± 20
PE_0.5	149.2	0.5	225 ± 28	8.9 ± 0.4	11.4 ± 0.4	8.6 ± 0.5	34 ± 8
PE_1.0	300.5	1.0	225 ± 34	8 ± 1	11 ± 1	8 ± 1	38 ± 8
PE_2.0	599.4	2.0	207 ± 35	7 ± 2	10 ± 1	7 ± 2	18 ± 2

10 mm/min, following the ASTM D628 standard procedure.

Hollow polymer particles were embedded in molten state LDPE matrices, aiming at the development of dispersed micro domains, acting as high deformable, load absorbing regions. Moreover, hollow domains are expected to act as discontinuity points in the polymer matrix, therefore significantly modifying the mechanical behaviour of the samples.

The mechanical behaviour of the prepared formulations was investigated by tensile test measurements, and compared to that of PE processed at 140 °C in the absence of micro particles. Results are reported in Table 2, and trends of E-Modulus and elongation at break at increasing micro particle content are shown in Figure 3.

As the hollow micro sphere content increases, the E-Modulus slightly decreases, (Figure 3a) reaching around 205 MPa for the 2% loaded sample The elongation at break significantly decreases, (Figure 3b) dropping down to 18% for the 2% loaded sample (Figure 3).

Sample morphologies were investigated by SEM. The addition of hollow micro spheres in the LDPE matrix resulted in a porous micro structure (Figure 4).

As the filler content increases, a more homogeneous distribution of cavity sizes within the polymer matrix was found. Interestingly, sample PE_0.5 (Figure 4b) showed the presence of 50–100 μm diameter cavities, likely due to the collapse of non-homogeneously distributed hollow spheres aggregates.

The cytotoxicity of LDPE based formulations loaded with hollow microspheres was investigated using NIH 3T3 murine fibroblasts (Figure 5). In all cases, cell morphology and spreading was found consistent with a not significant material toxicity.

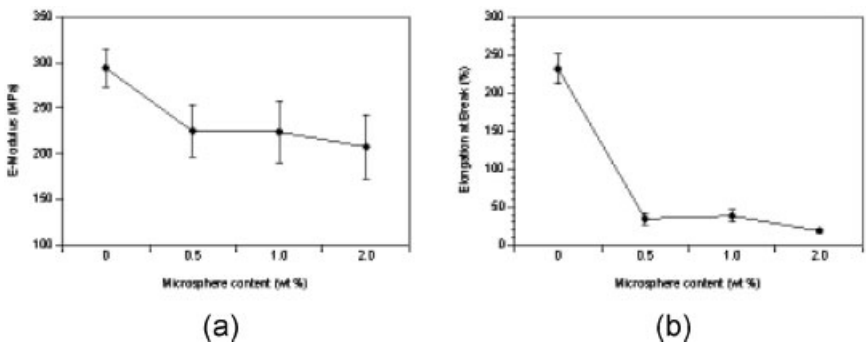

Figure 3.
E-Modulus (a) and elongation at break (b) trends for hollow micro spheres loaded PE based formulations

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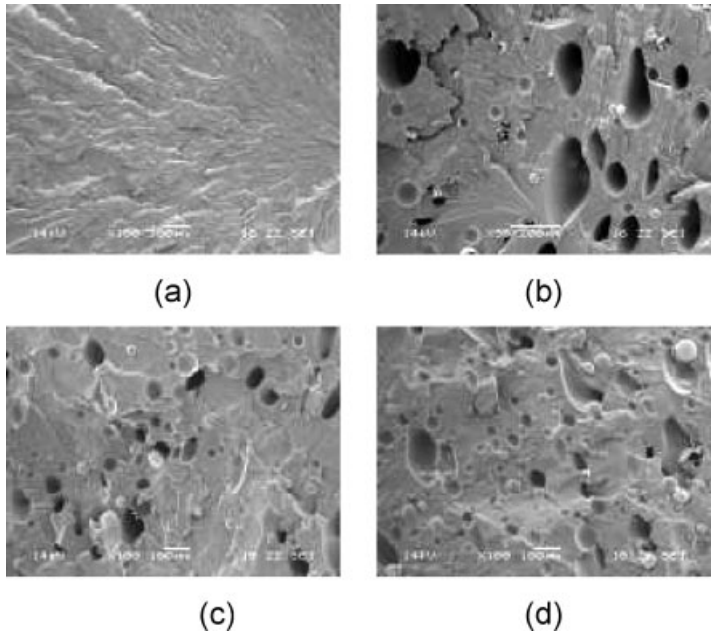

Figure 4.
Morphology aspect of LDPE matrix (a), and 0.5 wt % (b), 1 wt % (c) and 2 wt % (d) hollow spheres loaded LDPE formulations.

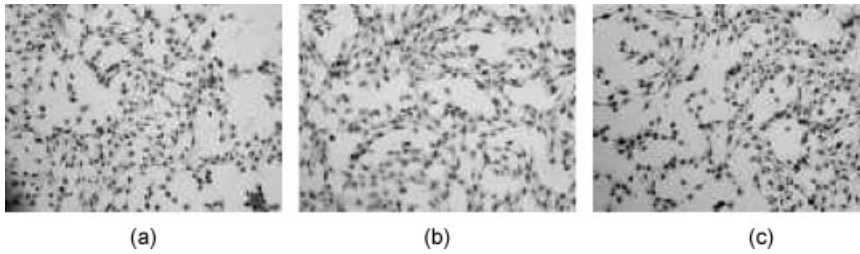

Figure 5.
Citotoxicity tests on LDPE based formulations containing 0.5% (a), 1% (b) and 2% (c) of hollow microspheres.

Conclusion

GG hydrogel thank to its thixotropic behaviour could be used in some medical device as in the nucleus pulposus replacement. The detection by AFM of nanometric particles of which the hydrogel resulted to be made, and SEM analysis are further evidences of its thixotropic nature. These data suggest that GG thixotropic nature is mandatory for the injection of the material into annulus fibrosus in the case of nucleus pulposus substitution. In fact the material once injected, needs to regains its original consistence. Moreover, in the case of nucleus pulposus replacement, the recovery of its consistence should guarantee the same mechanical properties of the native material of fundamental importance for the spine. In the perspective of total disc replacement, suitable formulations for the *annulus*

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fibrosus were developed, by means of tailored dispersion of hollow micro particles in commercial LDPE matrices, resulting in low toxicity, porous materials with modulated mechanical behaviour.

[1] L. M. Boyd, Andrew J. Carter, Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc. *Eur Spine J* **2006**, *15* (Suppl. 3), 14–21.
[2] J. Noailly, H. J. Wilke, J. A. Planell, D. Lacroix, How does the geometry affect internal biomechanics of a lumbar spine bi-segment finite element model? Consequences on the validation process. *Journal of Biomechanics* **2007**, doi:10.1016/j.jbiomech.2006.11.021.
[3] H. Freundlich, F. Juliusburger, Thixotropy, influenced by the orientation of anisometric particles in sols and suspensions. *Trans. Faraday Soc.* **1935**, *31*, 920–921.

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