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Design and characterisation of hydrogels and cryogels for low back pain treatment

Tesi in

Fenomeni di trasporto

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To my mum and dad.

Questo testo è stato stampato in proprio, in Times New Roman.

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Abstract

The main goal of this work was to estimate the performance of two gel – like materials, hydrogels and cryogels, in order to let them replace the inner part of the intervertebral disc, the nucleus pulposus. These materials are suggested to be a resolution for the Disc Degenerative Disease, a diffused problem related to the loss in mechanical properties of the intervertebral disc. At the early stage this pathology appears as a loss in hydration of the nucleus pulposus, that is the reason why its renewal with an appropriate gel – like material would seem resolutive.

Gravimetric, rheological and mechanical analysis were conducted on two kinds of gels: hydrogels were prepared as covalently gels of Hydroxyethylcellulose (HEC) crosslinked with Divinyl Sulphone (DVS), in order to replace the human degenerate nucleus pulposus, cryogels were made with Hyaluronic Acid (HA) chemically crosslinked with Ethylene glycol diglycidyl ether (EGDE), to create a scaffold for the implant of stem cells.

Hydrogels were prepared in three HEC/DVS ratios: 8/1, 4/1 and 2/1, and three diameters moulds were used: 5, 12 and 20 mm.

Hydrogels swelling kinetics was studied in water; rheological tests were conducted with the help of a plate – plate rheometer; stress – relaxation analysis was performed on hydrogels in air for 600 s.

Since the inner part of the intervertebral disc is characterised by an elevated degree of hydration, gravimetric tests allowed to estimate the swelling kinetics of the prepared gels: swelling equilibrium for hydrogels is related to the crosslinker amount, the higher the crosslinker amount the lower the water uptake.

In order to mimic the behaviour of human Nucleus Pulposus mechanical tests were performed. Frequency sweeps tests were done

on HEC/DVS hydrogels: storage and loss moduli were collected for all the types of hydrogels. The values of G' were three orders of magnitude higher than G'', defining the hydrogels' network as an elastic one. G' and G'', obtained as functions of frequency ω, were manipulated through the Generalised Maxwell model and a statistical procedure to obtain data in terms of time scale, confirming the prevalent elastic nature of the system.

Stress- relaxation analysis was led and results were displayed, highlighting the compression and relaxation phases separately. From the compression part of the analysis it was possible defining the maximum stresses and Young elastic moduli trends with strain percentage and initial polymer mass fraction: the higher the DVS amount the higher the influence of the strain on the maximum stress. The DVS has also an impact on the Young modulus, being E higher at lower HEC/DVS ratio. These values of elastic moduli are comparable with the data collected through the analysis of human and animal nuclei. The relaxation phase of the analysis was displayed in terms of stress versus time plots defining a dependence on the HEC/DVS ratio, on the strain and on the diameter: the higher the crosslinker quantity the earlier the relaxation shows; the higher the strain imposed to the specimen the more evident the relaxation; the smaller the gel the higher the relaxation. These behaviours resembled the idea of hydrogels' poroelastic nature, based on the migration of water and, in the latter case of dimension dependence, of the characteristic time of diffusion, directly proportional to the square of the radius. Experimental Young moduli values were compared with the values of unrelaxed shear modulus obtained from the fitting of rheological parameters, showing that $E = 3G_{un}$, typical of an elastic solid.

Cryogels obtained, had 10 and 20 mm diameters.

Gravimetric analysis was conducted on cryogels testing them in water and in a sugar solution at 83% concentration of syrup. Syrup solution was chosen as a swelling medium in order to mimic the increase in viscosity when systems are implanted with cells. Tests of stress – relaxation were done on cryogels in air for 600 s.

Cryogels in water swelled fast, imbibing an amount of water that was almost six times the polymer mass fraction instantaneously; cryogels were soaked in sugar solution to let the sugar penetrate the porous network and reach the equilibrium state.

Mechanical results for cryogels were treated as hydrogels. Compression step, for both gels swollen in water and syrup solution, shows trends with the swelling media, cryogels in syrup have higher stress peaks than in water, and with the mass fraction, the higher the mass fraction, the higher the stress peaks. Relaxation was described by stress – time graphs: cryogels in water shown a poroelastic migration of water, stress decreased continuously during the test; cryogels in sugar exhibited a previous strong stress diminishing until a certain minimum value was reached and remained constant to the end of the analysis probably for an interaction between the macroporous crosslinked gel structure and the syrup solution, the viscosity of the sugar system shows a higher viscosity than water swollen cryogels so that the minimum value reached by cryogels in syrup is higher than the ones’ in water because of the difficulty in water migration. This latter behaviour is the qualitatively closest to the *in vivo* tests results on human Nucleus Pulposus.

Sommario

L'obiettivo principale di questo lavoro è stato la valutazione delle performance di due materiali, hydrogel e cryogel, per sostituire la parte intera del disco intervertebrale, il nucleus pulposus. Questi materiali sono proposti come soluzione alla Discopatia Degenerativa, un problema legato alla perdita nelle proprietà meccaniche del disco. Agli stadi iniziali la patologia si presenta come una perdita dell'idratazione del nucleus, motivo per cui il rinnovamento dello stesso con un materiale di tipo gel appropriato sembra risolutivo.

Analisi gravimetriche, reologiche e meccaniche sono state condotte su due tipi di gel: gli hydrogel sono stati preparati come gel covalenti di Idrossietilcellulosa (HEC) reticolata con Divinil Sulfone (DVS), per sostituire il nucleus pulposus umano degenerato; i cryogel sono stati preparati con Acido Ialuronico (HA) reticolato chimicamente con Etilene glicol diglicidil etere (EGDE), in modo da creare una struttura scaffold per l'impianto di cellule staminali.

Gli hydrogel sono stati preparati in tre diversi rapporti HEC/DVS: 8/1, 4/1 e 2/1, e sono stati impiegati stampi cilindrici di tre diametri: 5, 12 e 20 mm.

La cinetica di swelling degli hydrogels è stata studiata in acqua; test reologici sono stati condotti con l'ausilio di un reometro di tipo piatto – piatto; analisi di stress – rilassamento sono state condotte su campioni in aria con durata di 600 s.

Essendo la parte centrale del disco intervertebrale caratterizzata da un elevato grado di idratazione, test gravimetrici hanno permesso di stimare la cinetica di swelling dei campioni preparati: l'equilibrio di swelling per gli hydrogel è legato alla quantità di reticolante presente in essi, più alta è la quantità di reticolante più bassa è la quantità di acqua assorbita.

Al fine di simulare il comportamento del nucleus pulposus umano, sono stati condotti test meccanici. Gli hydrogel sono stati sottoposti a test di frequency sweep: moduli di accumulo e perdita sono stati condotti su tutti i tipi di campioni. I valori di G' sono tre ordini di grandezza maggiori di G'', descrivendo il sistema come elastico. G' e G'', ottenuti come funzione della frequenza ω, sono stati manipolati attraverso il Modello di Maxwell Generalizzato ed una procedura statistica per ottenere i dati in termini di scala temporale, confermando la natura prevalentemente elastica del sistema.

Le analisi di stress – rilassamento sono state portate avanti ed i risultati mostrati, descrivendo le fasi di compressione e rilassamento separatamente. Per quanto riguarda la compressione è stato possibile definire gli andamenti di stress massimi e moduli elastici di Young con la percentuale di deformazione e la frazione massica iniziale di polimero: maggiore è la quantità di DVS maggiore è l'influenza della deformazione sullo stress massimo. Il reticolante ha anche un impatto sul modulo elastico, essendo E più alto a più bassi rapporti HEC/DVS. I valori dei moduli elastici sperimentali si sono dimostrati completamente comparabili con i valori di E descritti dalle analisi di stress – rilassamento non confinate condotte su nuclei umani ed animali. La fase di rilassamento è stata mostrata in termini di grafici di stress contro tempo presentando una dipendenza dal rapporto HEC/DVS, dalla deformazione e dal diametro: più alta è la quantità di reticolante prima appare il rilassamento; più piccolo è il gel più alto è il rilassamento. Questi comportamenti rappresentano l'idea della natura poroelastica degli hydrogel, basata sulla migrazione dell'acqua e, nel caso della dipendenza dalla dimensione, del tempo caratteristico di diffusione, direttamente proporzionale al quadrato del raggio. I valori del modulo di Young sperimentali sono stati comparati con i valori del modulo di unrelaxed shear ottenuti dal fitting dei parametri reologici, dimostrando che $E = 3G_{un}$, tipico di un solido elastico.

I cryogel sono stati formati con 10 e 20 mm di diametro.

L'analisi gravimetrica è stata condotta testando i cryogel in acqua e in una soluzione di sciroppo all'83 % di concentrazione. La soluzione di sciroppo è stata scelta come mezzo di swelling in modo da simulare un aumento della viscosità quando questi sistemi sono inseminati con cellule staminali. Test di stress – rilassamento sono stati condotti con cryogel in aria per 600 s.

I cryogel in acqua si rigonfiano velocemente, assimilando una quantità di acqua circa sei volte la frazione massica istantaneamente; i cryogel sono stati immersi nella soluzione di sciroppo al fine di far penetrare lo zucchero nel network poroso e raggiungere lo stato di equilibrio.

Risultati meccanici per i cryogel sono stati trattati come per gli hydrogel. Lo step di compressione, per entrambi i gel in acqua e sciroppo presentano trend con i mezzi di swelling, i cryogel in sciroppo presentano stress di picco più alti che in acqua, e con la frazione massica, più alta è la frazione massica, più alti sono gli stress massimi. La fase di rilassamento è stata descritta mediante grafici stress – tempo: i cryogel in acqua presentano una migrazione di acqua di tipo poroelastico, lo stress diminuisce continuamente durante il test; cryogel nella soluzione di zucchero mostrano uno stress che diminuisce fino ad un certo valore minimo e rimane costante fino alla fine dell'analisi probabilmente a causa di un'interazione tra la struttura macroporosa reticolata e la soluzione di sciroppo; la soluzione di zucchero presenta una viscosità più elevata rispetto all'acqua, per cui il valore di stress minimo raggiunto in sciroppo è maggiore a causa della difficoltà di migrazione dell'acqua. Quest'ultimo comportamento è qualitativamente più vicino ai risultati di test *in vivo* sul nucleus pulposus umano.

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“Faccio sempre ciò che non so fare per imparare come va fatto.”

Vincent Van Gogh