The Effect Of Modified Hyaluronan Hydrogel On The Prevention Of Epidural Fibrosis - In Vitro Cell Culture And Rat Laminectomy Model

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Introduction: Laminectomy has been used to explore the spinal canal and decompress the neural elements in spinal surgery. However, postlaminectomydural adhesion sometimes causes symptomatic problems and leads to difficulties in re-operation if needed. Therefore, prevention of peridural fibrosis and adhesion is important for successful laminectomy. Several materials have been used in vitro or in vivo as space occupying agents at surgical sites, and the results have generally been inconsistent. In our previous study, an injectable oxidized hyaluronic acid / adipic acid dihydrazide (Oxi-HA / ADH) hydrogel with good biocompatibility was developed. It can maintain its gel-like state for at least 5 weeks with a degradation percentage of 40% which seemed a good candidate to stop ingrowth of fibroblast from surrounding paraspinal muscles in the golden healing period at the laminectomy site

Methods: Preparation of Oxidized Hyaluronic Acid
Hyaluronic acid (HA) with a concentration of 1%(w/v) was dissolved in double-distilled water at room temperature and then sodium periodate (NaIO4, 2.67%) was gently added under stirring. The molar ratio of NaIO4 to HA was 1:1.
Preparation of Hyaluronic Acid-ADH Hydrogel
Dissolved the different NaIO4-ratio (6% w/v) oxi-HA and (8% w/v) adipic acid dihydrazide/ADH in PBS solution separately.
Cell
Cell line of fibroblasts (NIH 3T3), myoblasts (C2C12), Schwann cell (RSC96) and neural Cells (PC 12) was chosen for the biocompatibility studies of oxi-HA/ADH hydrogel.
Measurement of Cellular Proliferation
An amount of 5x103cells (PC12 & NIH3T3) and 2.5x103cells (C2C12 &RSC96) were seeded into a well of a 96-well microplate and then incubated in 0.2ml medium for 72 hours. Cellular proliferation in each well were determined by WST-8 assay. Absorbance at 450 nm was measured using a microplate absorbance reader. Cell counts were determined with a calibration curve of the tested cell type.
Measurement of cytotoxicity
An amount of 5x103cells (PC12 & NIH3T3) and 2.5x103cells (C2C12 & RSC96) were seeded into a well of a 96-well microplate and then incubated in 0.2ml medium for 72 hours. LDH released in the medium was quantitatively assessed by spectrophotometer readout at 490 nm. Percent cytotoxicity was expressed.
Animal experiment
3-month-old Wistar rats will be used in this study. Under anesthesia by intra-abdominal injection of sodium pentobarbital (30mg/kg), laminectomy with a longitudinal length of 10 mm and transverse width
of 5 mm at thoracic, and lumbar area in each rats using mini-rongeur under microscope was performed. In study group, hydrogel was used for laminectomy site coverage; in control group, we did laminectomy only without any material coverage.

MRI

In 8 weeks & 16 weeks after surgery, 7T-MRI was arranged for evaluation of morphology at fibrotic tissue overlying exposed dural sac..

Biochemical analysis

In 16 weeks post-surgically, animals were euthanized by intra-abdominal injection of overdosed sodium pentobarbital. The spine was cut into an complex including one above and one below segments around the laminectomy level for gross analyses, histologic analyses and immunohistochemical staining. Statistical analysis: Wilcoxon matched-pairs signed ranks test was used to evaluate the values in each parameters between the control and treatment group. Statistical significance was considered at p < 0.05.

Results: In cell proliferation measurement, we found that in NIH3T3 cell, cell cultured in hydrogel medium showed slightly lower proliferation rate. Cytotoxicity measurement showed rate of cytotoxicity of PC12, C2C12 and RSC96 cell was comparable when cultured in hydrogel and standard culture medium. In NIH3T3 cell, cell cultured in hydrogel medium showed higher toxicity with significance (Fig. 1). Histological analysis showed no arachnoidal adhesions in any case, but a significant difference was found between the treated and control defects in terms of the extent of postoperative epidural fibrosis and dural adhesion (Fig 2: control; Fig 3: hydrogel). There were similar findings on MRI which showed all control sites had relative dense epidural fibrosis with rich neovascularization, signs of chronic inflammation, and marked dural adherence to a various extent.

Discussion: Hydrogel has the advantages of high water content, good flexibility and effectiveness as a sustained-release drug delivery system. Clinically, exposed dural sac or neural tissue was surrounded by irregular and uneven bony surface at laminectomy site. So an ideal material may be an injectable biodegradable hydrogel with good biocompatibility, which is liquid in room temperature for easy coverage of rough bone surface and then gelated in body temperature in few seconds. The degradation time should be around 6 weeks that activity of fibrous connective tissue ingrowth is highest in the meantime.

In this study, we found that this injectable oxidized hyaluronic acid / adipic acid dihydrazide (Oxi-HA / ADH) hydrogel showed good biocompatibility. It doesn’t lead to the excess cell death in myoblast, fibroblast, Schwann cell and neural cell. Cell proliferation is good in cells except for fibroblast (NIH3T3), indicating that inhibition of fibroblast proliferation may cause possible less scar tissue formation in hydrogel covered surgical site. It is proved that this hydrogel was able to decrease the tenacity of adhesion at an interface between the dura and epidural scar. This result was shown not only by the gross and histologic analysis ranking scores but also by the MRI image findings. These findings may suggest the advantages of applying the new agent at a laminectomy site to minimize postoperative complications.

Significance: injectable oxidized hyaluronic acid / adipic acid dihydrazide (Oxi-HA / ADH) hydrogel can play a role in postlaminectomy epidural fibrosis prevention
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