Developing a Minimally Invasive Nucleus Replacement Procedure Using High Intensity Focussed Ultrasound (HIFU): Effect of Hyperthermia on Collagen Integrity and Cell Viability in the Intervertebral Disc

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Introduction: High-intensity focused ultrasound (HIFU) enables non-invasive heating of tissue volumes as small as a grain of rice located at a depth of 20 cm beneath the skin. HIFU is already successfully used clinically for non-invasive ablation of deep-seated solid tumours in the liver and kidney, as well as for treating uterine fibroids [1]. The technique has not so far been used to treat spinal discs although other minimally invasive techniques such as radiofrequency probes (Coblation® Nucleoplasty) are employed to ablate the nucleus pulposus for treatment of discogenic low back pain.

We propose employing HIFU as a new minimally invasive procedure for treating low back pain associated with disc degeneration via an injectable nucleus replacement. High intensity focused ultrasound (HIFU) is used to ablate the degenerated nucleus pulposus non-invasively, thus creating a void which is then filled with an injectable hydrogel to restore disc mechanical function.

As HIFU leads to a rise in temperature which could damage tissues adjacent to the treated areas, safe temperature regimes for HIFU treatment need to be determined. Here we examine the effects of hyperthermia on disc cell viability and matrix integrity at the temperatures and rates of heating typically encountered during HIFU therapy.

Methods: Bovine coccygeal discs were used as a source of intervertebral disc (IVD) to assess thermal damage to both cells and the collagen matrix. Discs were divided into outer annulus fibrosus (OAF), inner annulus fibrosus (IAF) & nucleus pulposus (NP), each region comprising about 1/3 of disc total area (Fig. 1,a). Both enzymatically isolated disc cells and cells embedded in tissue were heated in a water bath. The temperatures tested ranged from 40oC to 70oC for 10min. Cell viability of isolated cells after hyperthermal treatment was evaluated using CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega). Cell survival in tissue was assessed by methylthiazolyldiphenyl-tetrazolium bromide (MTT), which forms a dark blue precipitate in live cells that can be observed in bright field (BF) images. The tissue was stained with MTT after hyperthermal exposure, and cell nuclei were stained with DAPI and observed in fluorescent light (FL). Cells were classified as live when MTT staining overlapped with DAPI fluorescence, whilst dead cells exhibited DAPI staining only. Thermal damage of the collagenous matrix was assessed by examination under polarized light (PL). In addition, intact bovine discs were subjected to HIFU treatment to create a void in the centre of the disc (Fig. 1,b). Intact discs were dissected from a bovine tail, embedded within a degassed 3.5% agar gel and mounted in water tank at 37oC. The temperature profile was monitored by a thermocouple inserted into a disc tissue adjacent to the HIFU treated area. Cell survival and matrix integrity in disc tissue adjacent to the HIFU treated site were assessed histologically as described above.
**Results:** The fall in survival of isolated disc cells was significant for NP and IAF cells at 50°C (Fig. 2,a); survival was negligible at 60°C. Survival of OA cells in suspension decreased noticeably at 50°C and was significant at 60°C. By contrast, in tissue, all cell types demonstrated MTT staining after hyperthermal exposure at 50°C for 10 min. (Fig. 2,b). Collagen structure was well preserved in all specimens up to temperatures of 60°C but not at 70°C (not shown).

It was also found possible to create a thermally-induced void in the NP of the intact disc. Here we show results where the temperature at the HIFU focus reached 83°C (Fig. 3A). A photograph of a transverse plane through the NP with adjacent IAF tissue (Fig. A,a) clearly shows three distinct regions: a central void (red arrow), a dark blue area with live MTT-stained cells (blue arrow), and a light area with dead cells lacking MTT staining in the direction of the HIFU beam (black arrow). No substantial thermal damage of the collagen structure in the matrix adjacent to the void was observed (Fig. A,c), and birefringence of the collagen matrix was not affected (Fig. A,f). The boundary between the areas containing live and dead cells is well demarcated in bright field (BF) images (red line on figure 3A, b). Fig. A,d-c show enlarged views of this boundary region in BF, FL and PL. Live cells are seen to exhibit MTT staining (Fig. A,d) coinciding with DAPI (Fig. A,e, green arrows), whilst dead cells exhibit DAPI stain only. If the focal temperatures in the HIFU-treated disc did not exceed 54°C, most cells showed MTT staining and collagen birefringence remained intact (Fig. 3B, g&i respectively). The position of thermocouple is marked with a surgical suture (pink arrows). On enlarged images of the area highlighted on figure 3 B,h (Fig. 3B, g-l) only a few cells were identified as being dead (Fig. 3 B, k, red arrows) and no collagen damage was detected (Fig3. B,k).

**Discussion:** These early experiments demonstrate that non-invasive removal of the nucleus pulposus by HIFU is possible and would avoid the problem of implant expulsion encountered in other systems. However the effects of HIFU-induced hyperthermia require careful investigation. As previously observed for cartilage cells [2], our experiments showed that the cells in tissue specimens are more resistant to thermal damage than those in cell suspension. Furthermore, cells are affected at lower temperatures than those at which the collagen network is damaged (>70°C). It appears that temperatures of the order of 50°C for several minutes do not significantly affect cell viability in the disc and would be acceptable in the context of a HIFU disc replacement treatment. Nevertheless, the long-term recovery of cells exposed to HIFU-induced hyperthermia warrants further investigation.

**Significance:** Current surgical treatments for lower back pain associated with disc degeneration, are highly invasive and have low long-term success rates. The present work thus aims to develop a novel, minimally invasive therapy for disc replacement without the need for surgical incision.
Figure 2. Cell viability after hyperthermal treatment for 30 min in suspension (a) and in tissue specimens (b). Cells in tissue specimens were more resistant to thermal damage than those in suspension.
Figure 1. Schematic of experimental set-up. a) Discs divided into OAF, LAF & NB specimens were either digested enzymatically to isolate cells or were used directly in experiments. Cells in suspension or in tissues were subjected to hypoxic/ischemia and cell survival was assessed by appropriate methods. b) Tissue discs dissected from a bovine tail underwent HIFU treatment, then were stained with MTT and assessed histologically.
Figure 1. HIFU treatment of intact disc. A. NP area (transverse plane) after HIFU treatment at 59°C in the HIFU focus. (a) A photograph of the frozen specimen. (b,h) Micrographs of 50μm section in BF and FL, respectively. Red arrows indicate a void created by HIFU (a,c). Blue arrows indicate the area showing live cells stained with MTT and black arrows show light area of dead cells (d,f). The red line indicates a boundary between areas containing live and dead cells (b). (j,k). BF, FL and PL images of the enlarged boundary area highlighted on image (b). Green arrows (c) indicate areas of live cells which correlate with MTT staining.

B. IAF area ( sagittal plane) after HIFU treatment at 59°C in HIFU focus. (c) A photograph of the frozen tissue specimen. (d,h) Micrographs of 50μm section in BF and FL, respectively. Pink arrows indicate a surgical scar marking position of the annulus (e,g). (j) The same section in FL, background of collagen in HIFU focus is preserved. (k,l). BF, FL and PL images of the area highlighted on image (j). Nuclei of dead cells lacking MTT staining (k) are indicated with red arrows.

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