blood must occur sequentially in order to avoid recirculating the same blood. The total surface for gas exchange apparently is enough for efficient oxygenation and carbon dioxide elimination, although appropriate electrolyte needs to be added during bypass to maintain the acid-base equilibrium in the normal range.

The main advantages of this oxygenator are first, the low priming volume (in contrast with the large priming volume of fiber oxygenators), and second, the reusability of the device in contrast with the single use of fiber oxygenators. The device could be hooked up to any venous drainage, arterial infusion bypass circuit. Its main disadvantage is that you have to build and operate it yourself, and it may require considerable technical skill. Once the machine is operational, the cost of experimental bypass drops significantly, well below that of a conventional, hollow-fiber oxygenator.

CONCLUSIONS

The described oxygenator is able to perform efficient blood gas exchanges and could be used as an artificial lung. Also, our results prove that proper body oxygenation could be achieved by draining blood from the carotid artery, oxygenating it externally, and reinjecting it into the same vessel.

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Three-dimensional Culture of Human Nucleus Pulposus Cells in Fibrin Clot: Comparisons on Cellular Proliferation and Matrix Synthesis With Cells in Alginate

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Abstract: Regeneration of nucleus pulposus (NP) tissue may stop or reverse early intervertebral disk (IVD) degeneration. Cellular proliferation and matrix synthesis can be promoted by incorporation of cells and bioscaffolds. However, insertion of preshaped solid bioscaffolds may damage remaining IVD integrity. Fibrin clots can be introduced in a minimally invasive manner with polymerization in desired three-dimensional shape and retention of cells. In this study, we investigated the cellular proliferation and matrix synthesis of human NP cells in the fibrin clots in vitro. Monolayer-expanded cells were embedded in fibrin clot or alginate and were cultivated in vitro for 2 weeks. Increased DNA content and decreased expression of apoptosis stimulating fragment (Fas)-associated death-domain protein in fibrin scaffolds suggested higher cellular proliferation and reduced apoptosis. Superior proteoglycan synthesis was found in fibrin scaffolds. As expression of collagens I and X increased and SOX9 expression decreased, fibrin scaffolds tended to promote fibrotic transformation and inhibit chondrogenesis. Adjustments of fibrin preparations are needed to make it more suitable for IVD regeneration. **Key Words:** Fibrin—Intervertebral disk—Nucleus pulposus—Tissue engineering.

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Regeneration of nucleus pulposus (NP) tissues in the early stage of intervertebral disk (IVD) degeneration can theoretically stop or even reverse the degenerative process. Tissue engineering techniques offer a possibility of regenerating IVD tissue. Several types of bioactive scaffolds have been innovated in order to provide an optimal microenvironment for cellular migration, proliferation, and maintenance of appropriate phenotype (1,2). However, introducing a preshaped solid scaffold into the IVD has to create a window on the annulus fibrosus; this window can damage the remaining integrity of the IVD and can cause further degeneration. Therefore, an injectable biphasic biomaterial, one that is initially liquid and then becomes solid after its introduction into the nucleus space, may be an ideal bioscaffold for regenerating the IVD (3). Fibrin monomers derived from cryoprecipitate can be polymerized with thrombin to form a semirigid to rigid fibrin clot (4). Fibrin clots may provide chemotactic and mitogenic stimuli to promote cellular migration, cellular proliferation, and matrix synthesis (5). Fibrin clots can act as a space-filling agent and three-dimensional structures to support the implanted cells, to maintain a specific shape and structural integrity, and to direct growth and formation for adequate new tissue development. The fibrin clots can be polymerized in the desired three-dimensional shape, while simultaneously ensuring the retention of cells at the injection site (6). Fibrin clot is theoretically a good injectable biomaterial for regenerating the NP in an IVD. Fibrin clot can be made from autologous or homologous blood components; therefore, it is theoretically superior to other synthetic bioscaffolds in biocompatibility and biodegradability. The purpose of this in vitro study was to investigate the cellular proliferation and matrix synthesis of human NP cells in the fibrin clots in comparison to that in the alginate scaffolds.

MATERIALS AND METHODS

Preparations of fibrin scaffolds and alginate scaffolds

Under the regulation of the research ethical committee, human NP tissues were obtained aseptically from six adult patients with lumbar disk herniation. NP cells were first isolated and then expanded by monolayer culture. Human fresh-frozen plasma and cryoprecipitate were purchased from the Blood Donation Center, Taipei, Taipei City, Taiwan, as starting materials. After being completely thawed, the cryoprecipitate was centrifuged at 5000 rpm at 4°C for 12 min to obtain a fibrinogen concentrate. Approximately 5 mL of fibrinogen concentrate was retrieved from 100 mL of the cryoprecipitate. Thrombin solution was prepared by adding one volume of 400-nM $CaCl_2$ into nine volumes of fresh-frozen plasma to activate thrombin. Monolayer-expanded NP cells were suspended in sterile fibrinogen concentrate at a density of 2×10^6 cells/mL or in 1.2% alginate (Sigma, St. Louis, MO, USA) at a density of 1×10^6 cells/mL. One hundred fifty microliters of fibrinogen concentrate and 150 μL of thrombin solution were gently mixed in a 12-mm culture insert (0.4-μm pore size; Millipore, Carrigtwohill, Ireland) and set inside a 12-well culture plate (Orange Scientific, Braine-l'Alleud, Belgium). To polymerize the alginate scaffolds, 100 μL of 102-mM $CaCl_2$ solution was added into 300 μL of alginate solution in each insert, and the culture insert was immersed in 1 mL of 102-mM $CaCl_2$ solution. The total number of NP cells was 3×10^5 in each fibrin or alginate scaffold.

Assays

After the first and second weeks of cultivation, the total contents of DNA and sulfated glycosaminoglycan (sGAG) were measured for each experimental group. RNA extraction for quantitative reverse transcription-polymerase chain reaction (PCR) was performed in a sample from each group at the end of the second week. Real-time PCR was amplified for glyceraldehyde-3-phosphate dehydrogenase, type I collagen (col1a1), type II collagen (col2a1), type VI collagen (col6a1), type X collagen (col10a1), aggrecan (AGC), decorin (DCN), SOX9, and Fas-associating death domain (FADD) protein. For statistical analysis, Mann–Whitney *U*-test (DNA and sGAG contents) and Wilcoxon matched-pairs signed ranks test (mRNA expression) were used to determine the statistical significance between the fibrin and alginate scaffolds. At the end of 2 weeks, one sample from each group was fixed in 10% neutral buffered formaldehyde solution, embedded in paraffin, and then sectioned at a thickness of 6 μm. After deparaffinized in xylene and graded alcohols, the sections were stained with hematoxylin-eosin (HE) staining for histological examination.

RESULTS

DNA content was significantly larger in the fibrin scaffolds than in the alginate scaffolds at the end of the first (336 ± 76 ng vs. 234 ± 53 ng, $P = 0.025$) and second weeks (622 ± 133 ng vs. 356 ± 54 ng, $P = 0.006$). Content of sGAG was significantly higher in the fibrin scaffolds than in the alginate scaffolds after 1 week (10.7 ± 2.5 μg vs. 4.5 ± 1.3 μg, $P = 0.004$) and 2 weeks (26.9 ± 4.1 μg vs. 13.9 ± 2.8 μg, $P = 0.004$) of

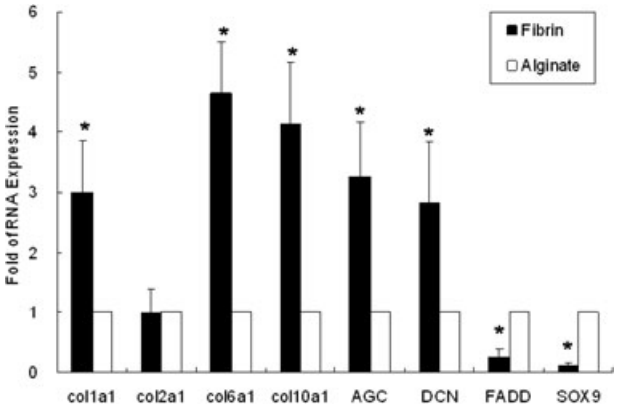

FIG. 1. Relative expressions of target genes in fibrin and alginate scaffolds were assessed with quantitative reverse transcription-polymerase chain reaction. Asterisks (*) indicate significant differences. AGC, aggrecan; DCN, decorin.

cultivation. Expressions of type II collagen were not significant in two tested scaffolds (Fig. 1). NP cells cultured in the fibrin scaffolds showed significantly higher expression of type I collagen (average: 300% ± 84, $P = 0.028$), type VI collagen (average: 465% ± 86, $P = 0.028$), and type X collagen (average: 412% ± 104, $P = 0.028$) compared with NP cells in the alginate scaffolds. For proteoglycans, NP cells in the fibrin scaffolds expressed significantly higher levels of aggrecan (average: 326% ± 91, $P = 0.028$) and decorin (average: 282% ± 101, $P = 0.028$) than those in the alginate scaffolds. In NP cells in the fibrin scaffolds, mRNA expression of SOX9 (average: 11.5% ± 4.9, $P = 0.028$) and FADD (average: 26.0% ± 13.8, $P = 0.028$) was significantly lower than it was for cells in the alginate scaffolds (Fig. 1). For sections obtained from the cell-scaffold hybrids cultured for 2 weeks, HE staining revealed clustering of cells along with abundant matrix deposition (Fig. 2).

DISCUSSION

Human NP cells cultivated in the fibrin clots manifested a more active proliferation, a lower tendency of apoptosis, and superior proteoglycan synthesis. However, significantly enhanced expression of types I and X collagens indicated a prominent tendency for fibrotic transformation in the fibrin scaffolds. In addition, expressions of "master chondroregulatory gene" SOX9, which regulates the expression of type II collagen and proteoglycans in IVD cells, suggested that chondrogenesis was poorer in the fibrin scaffolds than in the alginate scaffolds. The overall effects on matrix production and chondrogenesis with the current fibrin scaffolds seemed to be suboptimal for regeneration of human NP tissues. The suboptimal results were probably related to the initial cell density, the initial fibrin composition, and the cell type used in this study. To reflect the avascular environment and low cell density in the IVD, cell density used in the studies on culturing IVD cells is usually 1×10^6 cells/mL (7,8). This density is lower than that of articular chondrocytes cultivated in the fibrin clot ($10–40 \times 10^6$ cells/mL) (3,4,9). The initial density considerably affects the developing composition, structure, and function of the cell-scaffold construct (10). A high cell density helps to maintain phenotypes of the chondrocytes. The appearance and mechanical strength of the fibrin clots may vary enormously when the components and concentrations of fibrin composition differ (11). Therefore, it is extremely important to determine the exact matrix parameters necessary for a specific application. However, the exact contribution of each parameter is still not fully understood. Cells from different sources may also manifest differently in the fibrin clots. For instance, articular and auricular chondrocytes respond in different ways when they are cultivated in fibrin clots (12).

CONCLUSIONS

Fibrin clots promoted the proliferation of human NP cells and the synthesis of proteoglycans. However, the fibrin scaffolds also tended to promote fibrotic transformation and lower the probability of

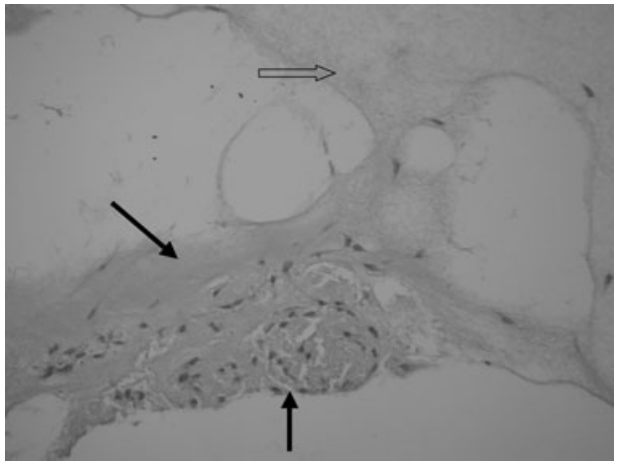

FIG. 2. After 2-week in vitro cultivation of human NP cells in the fibrin scaffold, HE staining revealed clustering of cells along with abundant matrix deposition. The newly synthesized matrix (solid arrows) around cell clusters was much denser than that in the original fibrin scaffold (open arrow) (original magnification: ×100).

chondrogenesis. Further adjustments of fibrin composition and initial cell density may be needed to make fibrin clots suitable for regenerating IVD tissues.

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Is Laterality Associated With a Higher Rate of Hip Arthroplasty on the Dominant Side?

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Abstract: The authors analyzed data collected by the Registry of the Orthopedic Prosthetic Implantology in Italy. They found a higher rate of total hip arthroplasty on the right side (58%) in comparison with the left side in patients affected by primary coxarthrosis. To test whether laterality was the cause of this, they checked the prevalence of the upper and lower limbs in 262 patients treated for monolateral total hip prosthesis. They found that the percentage of left-handed patients was very low (0.8–6.5%). The percentage of left-footed patients was, instead, 26.5% for power tasks. They observed that, while the rate of arthroplasties on the right side was similar to that of the left side (50.7 and 49.3%) in the right-footed patients, there was a clear-cut prevalence in the number of operations on the right hip (76.8%) in comparison to the left one (23.2%) in the left-footed patients. The authors suggested that, in left-footed patients, the right side was subjected to greater stress. **Key Words:** Hip prosthesis—Coxarthrosis—Laterality—Handedness—Left-footed.

Hip arthroplasty is mainly performed for the treatment of chronic pathologies, such as primary hip arthritis, sequelae of congenital diseases (dysplasia or congenital hip dislocation), and rheumatic arthritis.

In some countries, registers have been used for years to collect data related to primary operations and possible failures, to gather information on implants, surgical methodology, and characteristics of the patients, which mostly influence the result of the operation. In Emilia-Romagna, a region located in North Italy, with a population of around 4 500 000 inhabitants, a register of hip prostheses has existed since 2000 (Registry of the Orthopedic Prosthetic Implantology [RIPO]) and collects data on all the operations of total and partial hip arthroplasty and all revisions.

Analyzing the demographic characteristics of the patients present in RIPO, it was observed that, out of 29 697 primary operations, 16 543 (55.7%) were per-

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