Endovascular Treatment of Experimental Aneurysms by Use of Fibroblast-Coated Platinum Coils
An Angiographic and Histopathologic Study

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Background and Purpose—The purpose of this study was to determine whether implanting exogenous fibroblasts on platinum coils could enhance intra-aneurysmal fibrosis. Hypotheses included: (1) fibroblast-coated (FBC) platinum coils can improve angiographic results after embolization; and (2) FBC platinum coils can accelerate histological healing of embolized aneurysms.

Methods—Experimental aneurysms in rabbits were embolized with control platinum coils (n=18) or FBC coils (n=18). Subjects were euthanized at 14 days, 1 month, 3 months and 6 months after implantation. Digital subtraction angiography was used to evaluate stability after embolization. Histological samples were examined with a grading system (range, 0 to 12) based on neck and dome healing.

Results—Histology total scores and fibrosis ratio at 14 days were significantly greater in the FBC coil group compared with controls (6.6±1.9 versus 2.5±1.1, 1.2±0.6% versus 0.2±0.3%, respectively; P=0.0090). Cavities embolized with FBC coils showed cellular proliferation and thrombus organization, with an endothelialized membrane bridging the neck. There were no differences between groups in the later timepoints. The FBC coil group showed radiographic stability in 11 (61%) cases, coil compaction in 2 (11%) cases, and progressive occlusion in 5 (28%) cases. No progressive occlusion was seen in controls; 3 (17%) of 18 control cases exhibited coil compaction (P=0.0546).

Conclusions—FBC coils can accelerate early histological healing compared with control coils in the rabbit aneurysm model. (Stroke. 2007;38:170-176.)

Key Words: angiography ■ experimental aneurysm ■ fibroblast coated coil ■ histology ■ rabbit
Grading Scale for Elastase-induced Saccular Aneurysms

<table>
<thead>
<tr>
<th>Scale No.</th>
<th>Gross Neck</th>
<th>Micro Neck</th>
<th>Neck Compaction</th>
<th>Dome</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No coverage</td>
<td>Unorganized clot</td>
<td>Macro-concave</td>
<td>Blood clot (all)</td>
</tr>
<tr>
<td>1</td>
<td>&lt;50% coverage</td>
<td>Fibrin</td>
<td>Micro-concave</td>
<td>Organized tissue in &lt;1/3 of dome area</td>
</tr>
<tr>
<td>2</td>
<td>50% to 75% coverage</td>
<td>Fibrin with organized tissue</td>
<td>Flat</td>
<td>Organized tissue in 1/3 to 2/3 dome area</td>
</tr>
<tr>
<td>3</td>
<td>&gt;75% coverage</td>
<td>Completely organized tissue, thickness ≤1/3 of coil diameter</td>
<td>Convex</td>
<td>Organized tissue in &gt;2/3 of dome area</td>
</tr>
<tr>
<td>4</td>
<td>N/A</td>
<td>Completely organized tissue, thickness &gt;1/3 of coil diameter</td>
<td>N/A</td>
<td>Completely organized loose tissue</td>
</tr>
<tr>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Completely organized dense fibrous or cellular tissue</td>
</tr>
</tbody>
</table>

FBC Coil Preparation

The cells, cultured for seeding onto platinum coils, were obtained from explant samples of tendon as described by Evans and Trail. Briefly, portions of flexor tendon were harvested, stripped of their external sheath and cut into approximately 0.2×0.5 mm pieces. The pieces were rinsed in cell culture medium consisting of Dulbecco’s Modified Eagle Medium (DMEM F-12), Amphotericin B (5 μg/mL), penicillin (500 U/mL) and streptomycin (500 μg/mL); all media components were from Invitrogen. The pieces were then incubated in FBS alone for 10 minutes, then placed into T25 flasks containing medium and maintained at 37°C. After a confluent cell layer was obtained, the cells were trypsinized and diluted at 1.5×10⁴/mL in medium. Five milliliters of the medium with cells were placed in a culture dish containing platinum coils. Once cells had grown to confluence, the coils were removed and used for implantation. These cells were labeled with fluorescence Dil (Sigma-Aldrich Chemical Co.). Cells were also grown on slides for immunofluorescence studies.

Immunofluorescence in Cell Culture

Primary monoclonal antibody against human-a smooth-muscle actin (SMA) (Dako 1:200) and vimentin (Sigma-Aldrich, 1:40) were applied. After incubation with primary antibodies, the cells were rinsed with TBS and then incubated with secondary antibodies (Cy5-conjugated donkey anti-mouse IgG; FITC-conjugated donkey anti-goat IgG, Jackson Immuno Research Laboratories, Inc, West Grove, PA). Cells were viewed and imaged with fluorescence confocal microscopy. Ten random, high-power fields were imaged, and the number of total cells that were SMA positive and those that were vimentin positive were recorded. Positive controls included sections of tissue, which were positive for SMA or vimentin. Negative controls were performed with nonimmune normal serum used instead of the primary antibody.

Aneurysm Creation and Coil Embolization

Aneurysms were created in 36 female New Zealand white rabbits (body weight 3 to 4 kg) using the elastase-induced aneurysm model in rabbits. Eighteen subjects were embolized with standard platinum coils (controls) and 18 aneurysms were emboized using FBC coils. Methods used for embolization have been described previously. All animals were randomly assigned. Digital subtraction angiography was performed after embolization. Animals were permitted to recover and were euthanized at 14 days (control n=5, FBC n=5), 1 month (control n=3, FBC n=5), 3 months (control n=4, FBC n=5), and 6 months (control n=6, FBC n=3) after embolization. Aneurysm volume and coil packing density in both control and test group were calculated and compared using (Duration*Group interaction was not found then a Kruskal-Wallis test was performed on the control and experimental groups separately to examine differences within each group over time. When significance was found a Tukey-Kramer HSD test was performed to cull out any differences.

Tissue Harvest and Histological Processing

At euthanization, animals were deeply anesthetized. Digital subtraction angiography was performed, followed by euthanasia using a lethal injection of pentobarbital. Harvested aneurysms were immediately fixed in 10% neutral buffered formalin. The degree of gross tissue coverage was evaluated as previously described. Samples were embedded in paraffin and sectioned at 1000-μm intervals in a coronal orientation. Coil fragments were carefully removed. Sections were re-embedded in paraffin and sectioned at 5-μm intervals. Sections were stained with hematoxylin and eosin (H&E) and Masson Trichrome. Immunohistochemistry or immunofluorescence for SMA and vimentin was also used.

Angiographic Evaluation

All euthanization angiograms were compared with postembolization angiograms and assessed for changes in coil configuration or aneurysm filling. Euthanization angiograms were categorized as stable, progressive occlusion, or coil compaction/recanalization as compared with the post-treatment angiograms. A Fisher exact test was run on the angiographic data. Stable and progressive occlusions were categorized as good results, whereas compaction was deemed a poor result.

Histological Evaluation

Sections were viewed by 2 blinded and independent experienced reviewers paying particular attention to the thickness of the cellular layer across the neck of aneurysms and the cellularity within the aneurysm dome. An ordinal grading system (Table) was used to evaluate findings on H&E-stained slides. This scale was devised based on findings at the neck and in the dome. Neck healing was based both on gross and on microscopic inspection. Tissue coverage across the neck, as noted on gross inspection, in addition to microscopic findings, which included tissue thickness and tissue type, were graded. The scores of the gross and microscopic inspection were averaged to yield a single neck score. Microcompaction assessment was based on the shape of the coil mass across the neck, from convex to concave. Healing characteristics in the dome were categorized based on the density of cellular infiltration and area of organized tissue. These scores, neck average, microcompaction and healing, were added together to obtain a total score representative of the aneurysm’s pathology. A least squares test was performed on the variables to test for a Group*Duration interaction. If an interaction was not found then a Kruskal-Wallis test was performed on the control and experimental groups separately to examine differences within each group over time. When significance was found a Tukey-Kramer HSD test was performed.

Results

In Vitro Immunofluorescence

All cells in culture displayed strong immunoreaction to vimentin. Sixty percent of these cells strongly expressed SMA, indicating the majority of cells had obtained a smooth-
muscle cell phenotype during cell culture to become differentiated myofibroblasts.

**Angiography**

**Aneurysm Volume and Coil Density**

Mean aneurysm volume, determined by assuming the aneurysm is a cylinder and using the formula \( \pi r^2 h \), was 0.07 ± 0.05 cm³ and 0.10 ± 0.07 cm³, respectively, and were not significantly different \((P=0.1945)\). Mean coil packing densities in the control group and the test group were 34 ± 13% and 23 ± 8%, respectively \((P=0.0108)\). That is, packing densities in the control group were significantly higher than those in the treatment group.

**Control Group**

Coil compaction was noted in 2 of 5 subjects harvested at 2 weeks, 1 of 4 subjects at 3 months. The remaining 15 aneurysms showed stable occlusion \((P>0.05)\). There were no differences in coil compaction between the 2 groups. Both stable and progressive occlusion were considered negative. There were no changes in histology between the 2 groups.

**Test Group**

One aneurysm at 1 month, 3 aneurysms at 3 months, and 1 aneurysm at 6 months showed progressive occlusion. Coil compaction was observed in 1 of the 5 aneurysms at 1 month, and another one at 6 months. All other aneurysms showed stable occlusion \((P>0.05)\). Collagen deposition remained sparse in all 2-week and 1-month specimens. Localized collagen deposition was noted in the dome of 1 of 4 aneurysms harvested at 3 months, and 4 of 6 aneurysms at 6 months.
throughout the dome (Figure 3A). The necks of 4 aneurysms had dense spindle cell infiltration with a covering endothelial cell monolayer. One of 5 subjects had diffuse inflammatory cells that penetrated the aneurysm dome and a thin layer of neointima along the neck. Immunofluorescent images displayed the presence of Dil-labeled cells that were mostly positive for SMA staining (Figure 3B), indicating that implanted cells had maintained their myofibroblast phenotype. Immunohistochemistry and immunofluorescence showed spindle cells that were strongly positive for SMA and vimentin (Figure 3C and 3D), indicating the cells as well were differentiated myofibroblasts. The widespread collagen matrix deposited among spindle cells and within the cytoplasm of the cells was identified by Masson Trichrome stain.

One month after embolization, 1 of the 5 test aneurysms had organized connective tissue completely filling the dome, in addition to a thick, hypercellular tissue traversing the aneurysm neck. The other 4 specimens in the group had loose connective tissue that partially filled the dome, whereas a neointimal layer in conjunction with fibrin crossed the necks. At 3 months, 2 of 5 test-group subjects had dense, diffuse fibrous tissue within most of the aneurysm dome. Masson Trichrome staining showed collagen bundle formation.

At 6 months, 1 aneurysm dome was filled with dense connective tissue, and another 2 were filled with loose connective tissue. Organized connective tissue covered the aneurysm neck in 2 of the 3 aneurysms. The other aneurysm had connective tissue and fibrin across the neck. Macrocompaction was shown in 2 of the 3 aneurysms. Localized collagen deposition was noted in the dome in 2 of the 3 aneurysms harvested at 6 months.

**Semiquantitative Histology**

Mean histological scores in the control group at 2 weeks, 1, 3 and 6 months after embolization were 2.5±1.0, 3.1±2.5, 5.4±1.4, and 6.8±1.6, respectively. Scores in the test group at 2 weeks, 1, 3 and 6 months after embolization were 6.6±1.9, 4.7±2.8, 7.4±2.5, and 6.7±2.0, respectively. Mean histological score in control group at 6 months was significantly greater than that at 2 weeks and 1 month (P=0.0125). Histological healing in the test group at 2 weeks was better than that of control group at the same time point (P=0.0090; Figure 4). There were no other significant differences.

**Discussion**

In this study, we build on previous preclinical data to demonstrate that exogenous fibroblasts can be implanted into experimental aneurysms using platinum coils. Angiographic outcomes are excellent after FBC implantation, with no evidence of recanalization. At an early timepoint, namely 2
weeks after implantation, histological healing is improved in the FBC coil group compared with controls. This study offers new information in the aneurysm healing process. First, the study incorporates the implantation of labeled cells into a model of aneurysm healing. The dual-stained aneurysm samples show not only that the implanted fibroblasts survive the process of delivery, but also that they express contractile proteins, which are considered relevant for aneurysm healing. At both 2 and 4 weeks after coil implantation, control aneurysms still displayed unorganized thrombus, whereas FBC-treated specimens developed dense, cellular infiltration. The aneurysm cavity was filled with connective tissue at 3 and 6 months in both control and FBC-treated group. Although there was no statistical difference at the later time-points, control samples had loose connective tissue and local collagen in the dome, whereas FBC aneurysms developed a diffuse, collagen-rich matrix, densely infiltrated with nucleated cells. More collagen deposition within aneurysm was found at 6 months after embolization in control group. The ordinal scale that assessed aneurysm healing, although rudimentary and not yet validated, revealed differences between test and control cases during early healing. All of these findings suggest that cell implantation therapies may one day enable the complete sealing of cerebral aneurysms.

The authors previously reported preliminary data that exogenous cells can accelerate thrombus organization in embolized aneurysms,12,13,20 in a small number of subjects over short duration. The current study demonstrates that cell-bearing coils may improve histological outcomes at early timepoints.

Other authors have proposed cell implantation for improvement of aneurysm occlusion. Raymond et al21 demonstrated, using a canine model of gelfoam implants, that ex vivo growth of activated smooth muscle cells onto embolic devices improved aneurysm fibrosis. There are several important differences between this study and that of Raymond’s group. First, the experiments reported by Raymond et al21 surgically implanted gelfoam sponges, whereas in this study endovascular cell delivery was performed with standard microcatheters and modified, commercially available platinum coils. Second, Raymond et al21 implanted smooth muscle cells into experimental aneurysms, whereas this model implanted FBC. There are several theoretical advantages of using fibroblasts rather than smooth muscle cells. Fibroblasts rapidly proliferate in culture, are extremely hardy and thus are very frequently used for ex vivo gene therapy and cell transplantation work.22–25 Fibroblasts are already approved for human implantation. A tissue-engineered, Food and Drug Administration–approved, artificial skin containing fibroblast allografts harvested from neonatal skin samples has been available for treatment of nonhealing ulcers and other cutaneous defects.25–29 Little or no deleterious immune response to the implanted cells has been documented with this device.29 Recently, investigators from Japan30 and South Korea31 also reported research related to FBC coil embolization. Matsumoto et al30 used a rat model; a FBC coil was inserted into the common carotid artery, without an aneurysm. Kwon et al31 used a canine sidewall carotid aneurysm model in which a portion of autologous jugular vein was made into a pouch and stapled around a slit in the carotid artery wall. This model is problematic in 2 ways. First, it is potentially a model of vessel wall healing in response to a cut made in the artery wall rather than an aneurysm. Second, the compliant venous wall responds to flow and remodels differently compared with a stiff artery wall. Though the data from that study are complementary to the data in the present study, the saccular aneurysm model used here would seem a more realistic model with which to study aneurysm healing. Further, we report both angiographic and histological findings as well as

Figure 3. Photomicrographs of a single aneurysm from the 2-week group. Panel A demonstrates dense, diffuse spindle cell infiltration through the aneurysm dome (H&E; magnification=100×). Panel B illustrates Dil-labeled cells (red) within the aneurysm dome, which also express SMA (blue; magnification=40×; oil). Panel C shows that spindle cells throughout the dome are positive for SMA (immunohistochemistry, magnification=100×). Panel D shows that spindle cells throughout the dome are also positive for SMA (red) and vimentin (green; immunofluorescence, magnification=40×; oil).
reporting on later timepoints than those reported in the study by Kwon et al.31

In this study, fibroblasts were used because such cells are readily transformed into smooth-muscle–like myofibroblasts. Myofibroblasts are the key cell type in wound healing, offering both the production of extracellular matrix as well as contractile properties. Although fibroblasts are a promising cell type, difficulty in harvest may limit widespread application. We anticipate, as have other groups,32 that circulating progenitor cells may represent the ideal candidate cell for treatment of aneurysms. These progenitor cells are easily harvested and expanded ex vivo.

There were no differences in coil compaction. Mean coil packing densities in the control group and the test group were 34±13%, and 33±8%, respectively (P=0.0108).

The present study has limitations. The FBC coils cannot be retrieved and repositioned, though this could be easily overcome if commercial groups become interested. The sample size in each group was small and unequal. In addition, the survival time after embolization was short when viewed clinically. However, the mortality rates in animal experiments always make it difficult to achieve equal sample sizes, and unfortunately the authors had limited resources for this study. Statistical power was examined and, with the exception of the 1 and 3-month total-score groups, significantly more animals (n≥15) would have needed to achieve statistical powers of 0.7 or 0.8. The 2-week data are the most powerful, and it appears that the required sample size tripling, at minimum, to alter the power at the other timepoints indicates that the meaningful data are found at 2-weeks.

Summary

FBC coils can increase the biological activity of plain platinum coils during aneurysm embolization at early timepoints. This technique accelerated embolization and thrombus organization in the rabbit model and may be of potential clinical use.

Figure 4. A comparison of total scores over time by treatment groups; data are represented as mean±SD. * indicates that mean control (blue solid bar) value at 14 days is significantly different from the mean 14 day FBC-treated (red hatched bar) value. In all cases significance was set at P<0.05 (Wilcoxon and then a Tukey post hoc test; JMP version 5.1, SAS Inc).

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Disclosures

None.

References

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