Stem Cell Treatment May Influence AAA Growth And Behavior

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Disclosures

• None

Porcine AAA Creation (Sus scrofus)

- Isolate Aorta
- Introducer sheath
- Balloon Angioplasty
- Collagenase 16000U
- Elastase 1000U


Development of a Porcine AAA Model

- Control
- Renal a.
- AAA

Day 7

- Loss of nuclei
- Migration of neutrophils

Elastin

- Degradation, fragmentation, fraying and unraveling elastic fibers

H&E

40X
**Microarray Analysis**

1wk (N=3), 2wks (N=6), and 4 wks (N=5)

- RNA → cDNA → Biotin-modified aRNA → 20,201 genes per array

**Inflammation**

- IL-1β

Week 0, N=3

Week 1, N=3

Week 2, N=6

Week 4, N=5

- *P < 0.01

**Extra-Cellular Matrix**

- MMP-9

Week 0, N=3

Week 1, N=3

Week 2, N=6

Week 4, N=5

- *P < 0.01

**Elastin**

Week 0

Week 1, N=3

Week 2, N=6

Week 4, N=5

**PET / CT Scanning**

Activity appears as an annulus (An.) in two scans (A and B) on postoperative day three illustrating aortic wall activity following abdominal aortic aneurysm formation. The area of increased activity adjacent to the aorta (U) represents FDC within the ureter.

**Total Aortic Activity: Ellipsoidal Volume of Interest**

- Mean Activity

Post operative day
MSC Harvest

• Bone marrow aspiration from iliac crest

![Image of bone marrow aspiration](image1.png)

MSC Isolation

• Ficoll-paque Separation

![Diagram of Ficoll-paque Separation](image2.png)

Mesenchymal Stem Cells/LentiGFP*

Transfection of 293 T cells -> Collection of supernatant -> Infection of MSCs

Labeling efficiency: 92±15%

![Images of Ficoll-paque separation and supernatant](image3.png)

MSC Implantation

• Direct injection of MSC into ventral wall of AAA

![Image of MSC implantation](image4.png)

Ferex Labeling of MSCs

• Produces signal drop out on MR
• >90% viability, phenotype verified by flow cytometry
• Intralysosomal localization verified by TEM

![Image of Ferex labeling and TEM](image5.png)

Preservation of BM-MSC phenotype on Fluorescence Microscopy

Ferex-labeled BM-MSCs --> positive for CD90 and negative for CD45

Merged images with cell surface antibodies (+CD90, +CD117, -CD45, & DAPI).

![Images of fluorescence microscopy](image6.png)
GFP Labeling of AAA treated with MSCs at 21d

In-vivo- MRI

Picosirius Red Labeled AAA treated with MSC at 21d

Blue-Green = New Collagen Deposition in MSC treated persists at 21d (Red = Mature Collagen)

New Collagen Deposition in Regions Populated with MSCs

Immunohistochemistry images of 14-treated group:
Left – Stained for anti-GFP in porcine AAA tissue
Right – Stained with Picosirius stain (new collagen in orange/green)

MMP-3

MMP-9

IL-1β

IL-10

[Graphs showing MMP-3, MMP-9, IL-1β, and IL-10 levels in untreated and treated AAA tissues over different time points and regions (Anterior vs Posterior).]
Conclusions

• Porcine model of AAA bears similarity to clinical aneurysms histologically and genetic expression
• MR in vivo localization of labeled MSCs demonstrates progressive migration circumferentially around AAA
• AAA regions where MSCs are localized demonstrate new collagen production with corresponding smooth muscle actin expression
• In AAA treated with MSCs qPCR data demonstrate blunting of inflammatory and matrix degradatory markers (MMP-3, -9, IL-1B) associated with experimental AAA formation