Reviewer's report

**Title:** Human amniotic fluid stem cell injection therapy for urethral sphincter regeneration in animal model

**Version:** 1  **Date:** 9 December 2011

**Reviewer:** Margot Damaser

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This study was designed to investigate if periurethral injection of human amniotic fluid stem cells (hAFSCs) can restore competent urethral sphincter in a mouse model. This topic is novel and timely as stem cells show promise for regeneration of the urethral sphincter and there is a search at present for the optimal stem cell population for this purpose. The use of hAFSCs for this purpose is underinvestigated even though they may yet show the greatest promise. The study is, for the most part, well designed. However, the methods are insufficiently described, statistical assessment of results is inadequate and must be improved, the discussion section is incomplete and English usage needs to be corrected throughout the entire manuscript. Nine Major Compulsory Revisions are listed below.

1. Much more detail is needed in the methods section, particularly with regard to:
   a. The sham operation. Since the abdominal approach to pudendal nerve transection is quite invasive, the sham operation needs to be thorough but careful not to injure the nerve. More details on these methods is needed.
   b. Injected cells -- what passage were they at the time of injection; how soon after pudendal nerve transection were they injected; were they sorted or differentiated prior to injection?
   c. Human nuclei-specific antibody. Needs to be specified since it doesn’t appear to be in Table 2. Table 2 needs to provide a column that matches the terminology used in the text of the manuscript. It also needs to provide catalog numbers for each antibody. It also needs to include all antibodies utilized in the study or it is incomplete.
   d. Nanoparticles. Much more detail is needed on the nanoparticles, including a definition of MNPs@SiO2 outside of the abstract. How do the nanoparticles get integrated into the cells? Do they have the same density in all cells? Can they be shed by the cells? How do they get into both daughter cells if the host cell proliferates? If cells differentiate do they retain the nanoparticles? Were the cells sorted so that only those with nanoparticles were injected?
   e. Anesthesia. What anesthesia was used for optical imaging and for cystometry/LPP?
   f. Optical imaging. How frequently were animals imaged before 10 days? What settings and resolution were used for optical imaging? More details of methods of correction for background and autofluorescence are needed.
g. Flow cytometry. More details on methods of flow cytometry are needed.

h. Tumorigenesis assay. Why were animals assessed 2 weeks after injection for tumorigenesis? This seems like not nearly long enough.

i. Number of animals. If 30 animals were used in the study, why was data collected on only 5 in each of 3 groups?

j. Comparison to sham. Methods states results were compared to those of “normal control.” Were these the sham animals or a different group? The sham to an invasive nerve transection can hardly be called normal control.

k. Statistics. An unpaired t-test is the wrong test for this study. An ANOVA of one sort or another ought to be used for multiple comparisons. Sham injured animals need to be included in the statistical comparisons. Statistical comparisons of ALL quantitative outcomes ought to be made including results of viability assay, gene expression (PCR), and flow cytometry. Statistically significant differences need to be indicated in the figures. Consultation with an expert in statistics ought to be considered.

2. Conclusions that a quantitative result from one group is higher or lower than from another group is meaningless in the absence of statistics. Statements to this effect in the Results section need to be deleted unless statistics for these outcomes is performed with the correct statistical test.

3. Data for in vivo cell identification at day 14 should be shown in the figure.

4. The optical imaging results suffer from low resolution. Thus they are not able to conclude if cells migrate outside the region of interest. Ex vivo imaging of specific organs can be done with higher resolution and would show if cells migrated to other organs or regions.

5. It appears from the data that there was a deterioration of closing pressure (CP) in sham injured animals with time. Was this a statistically significant difference? If so, what significance does it have?

6. It is doubtful that you can accurately measure pressure to within 0.01 cm water. Please report only significant digits in results.

7. In the Discussion section it is stated that “. . . while control group remained low” regarding leak point pressure (LPP) and CP results. However, the control group (actually sham-injured group) has the highest value. Please correct the discussion section text.

8. The discussion section is incomplete and insufficiently references studies from the scientific literature. At a minimum it also needs to include:

a. A discussion of acute vs. chronic stress incontinence models. SUI is a chronic condition but the authors used an acute model. It is likely the injected cells in this study preserved muscle rather than restoring it. Particularly in a nerve transection model this is an important distinction.

b. A discussion of likelihood of proper innervation of the striated muscle after a pudendal nerve transection

c. A discussion of cell differentiation and its relevance to this situation.
d. A discussion of cell migration and its relevance to this situation.
e. A discussion of the tumorigenic outcomes, particularly given that animals were assessed only at 2 weeks after cell injection.
f. A discussion of limitations of the study.

9. English usage needs to be corrected throughout the manuscript. A native English speaker should review the entire manuscript in its revised version.

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.