Meeting Minutes for the Presentation

- **Concern #1:**
  - Don't really understand why we are testing immersion in solution containing spheres alone vs. perfusing the heart with the spheres in addition to immersing the heart, which is what is done clinically.
  - Why just the immersion? Unlikely the H2S will penetrate well, needs to be contact through vasculature. Believe we could skip over those preliminary experiments and just have the experiment where we perfuse and immerse
  - **Response:**
    a. NaHS tends to escape/depleted over time. So we want to see if we maintain the concentration, will that maintain the effects, or if the heart somehow develops resistance to the effects of NaHS over time
    b. To prove the microspheres are actually doing what we want them to, which is to distribute the NaHS in a uniform manner over time and how it's distributing over an area of the heart.
  - **Response to the response**
    a. Don't believe that the H2S will be able to penetrate the myocytes, at least not enough to prevent ischemia-reperfusion injury, just do a series of initial experiments to determine the constant rate of release from the spheres
    b. Add right away H2S to the perfusant and get it right in the area where you want it because from the outside of the heart there is a barrier of endothelial cells and it's hard to overcome so there won't be a huge difference if you just immerse it in spheres vs. just UW
    c. It's more practical, since even in immersion you have to perfuse the heart with something, so you might as well do it with the spheres and if there IS an effect, you can dissect it and figure out whether the immersion has done it or the perfusion or both.
  - **Solution(?)**
    a. Start with the retrograde injection through aorta of the spheres instead of the initial experiments we were going to do, which would help out with the need to increase number of experiment (as mentioned below)
- **Concern #2:**
  - The number of experiment per experiment it's relatively low, difficult to find that number.
  - 3 experiments per group in the animal section is "horribly" too low. Too much variability when working with animals so it's unlikely at the end of 3 groups we'll be able to have a statistically relevant data or to even detect a difference in outcomes.
    a. Probably at least need 5-6 experiments or even more if looking at smaller differences. Obviously, it makes it a large experiment, but if we don't then it ends up as not a strong experiment.

- **Concern #**3
    - Are we trained to do this? Seems pretty sophisticated and requiring sophisticated skills.
    - Response:
        a. Contacted campus vet. Said he could physically show us how to remove heart
        b. Team is divided into couple groups: Microsphere formation and heart extraction
        c. Don't need the training on how to deal with live animals because we'll be working with dead ones
    - Response to response
        a. Need to work with live animals toward the end of the experiments where we assess the function of the heart. Means that we need to keep the rat alive as we are starting the surgery.
        b. The few minutes of having a technically dead animal/heart could make the difference between success and failure
- **Concern #4**
    - The heart of a rat is very small, so biopsies will destroy the functionality of the heart, won't be able to put it on the Langendorf afterwards.
    - Suggested 2 groups, one where we biopsy, and one where we don't biopsy and do Langendorf.
    - Creatine-kinase is measured not in cells but in blood
        a. It is normally in the cell anyways, so it's obviously going to be there
- **Concern #5**
    - Propose to assess concentration affects, then cross-linking, but cross-linking will affect sphere size, which will affect release rate and probably the concentration.
    - Probably should first optimize the cross-linkage then the concentration
- **Response**
    - We're actually doing concentration without microspheres

- **Typo in Specific Aim 1 Experiment 1**: We wrote 25mM then 40mM should both be 25mM
- **Remarks**
        a. Open abdomen, flush via Vena Cava using Heparin to anticoagulate the blood, then inject via vena cava some protection liquid like UW, then open chest right away (heart's still beating), must cool heart right away, add some more cardioplegia to get the heart at a good function after preservation time.
        b. Endothelial Cell walls are so thick that even H2S would have a hard time penetrating completely
        c. Number of myocytes we were going to use, if we don't use enough, we will not be able to see any difference

d. Why are we adding BRDU to culture medium, it's an agent typically used to look at cell perforation(?). It's expensive and a biohazard
e. Feasibility of isolating myocardiocytes. Important to know if epithelial cells will take up H2S
    i. Backup/complimentary approach: Test it with epithelial cell cultures to give us an idea of how much/if they take up any H2S
f. Aim 2 Exp1→ Why PBS, why not UW? Maybe test if there's a difference or use Krebs-Heinselate
g. Whenever you explant the heart, you must use/inject cardioplegia. Cooling is not the best way.