# List of changes to JoVE52450 ‘An Injectable and Drug-loaded Supramolecular hydrogel for Local Catheter Injection into the Pig Heart’

**Editorial comments:**

*Changes to be made by the Author(s):  
  
2. Please describe step 4.3.3.1 in more detail. How are the injection locations determined? Is it a pattern? What is done to release the specified injection amount? How is the sample prepared prior to injection? How is the sample injected? In a bolus or while moving the tip of the needle? Better define the criteria and how they are measured.   
  
Please keep in mind the the 2 page limit of highlighted protocol text as filming will be split between two locations.*

**Reply:** Step 4.3.3.1 has been described in more detail. Sample preparation has been described in point 4.3.1. Due to these changes, point 4.3.3 is now obsolete. We thank the editor for these remarks, as we think the procedure is now much clearer. To stay within the 2 page limit, point 3.4.2 has been split and we have partially removed the highlighting.   
*3. Are there any histology images the authors could show of the hydrogel in the heart tissue?*

**Reply:** Here, we have not chosen to go into detail on the analysis of the heart after sacrifice of the animals but focus on the actual hydrogel formulation and injection methodology. For histology images and more information after sacrificing the animals, please see Bastings and Koudstaal (*Adv. Healthcare Mater.* **2014**, *3*, 70–78).   
  
**Reviewers' comments:**  
  
**Reviewer #1:**   
*Manuscript Summary:*   
*The Authors tried to demonstrate the methodology of formulation and characterizations of a hydrogel, and it's use for drug delivery system in the field of regenerative medicine. The hydrogel was formed by non-covalent interactions in between poly(ethylene glycol) (PEG) and end-modified with ureido-pyrimidinone (UPy) moieties. When tested on the myocardial infraction area of pig heart, this hydrogel helps to release the drug in a controlled manner. This is academically and clinically a very valuable methodology, specifically in the field of regenerative medicine and drug delivery system in the heart.*

**Reply:** We thank the referee for his/her detailed review of our manuscript and shared interest in the methodology; we believe that the points raised by the referee are of great interest and have tried to address these here and in our revised manuscript.   
  
*Major Concerns:*  
*1. HCl was used to adjust pH of the solution, and for the in vitro experiments, the solution was gelated by neutralizing the gel with HCl. However, for in vivo condition, the pH was mainly adjusted from the natural pH of the tissue. It may vary from the cell to cell or tissues and so, it is important to add the right amount of HCl to prevent an overshoot in pH. I am wondering if there is any way of monitoring the pH. In addition, how can you estimate the amount of HCl needed for the in vivo settings?*

**Reply:** We agree with the referee that the right pH is essential to create a good gel. However, for the solutions to become solid, the pH should be lowered below 8. For the in vivo release, we only rely on the buffering capacity of the body. In this case, we do not add any HCl. We have not monitored the pH of the gel in the body, but extract the success of the experiment from the formation of a gel. To make this clearer, we have clarified in point 4.3.2 that a solution is used, and added that it will only form a gel after injection in point 4.3.3.1.  
  
*2. The Authors demonstrated the mRuby2 molecule as a model protein as a proof of principle of the methodology. I'm wondering if this procedure is applicable to deliver the growth factors like VEGF or EGF molecules and for how long they can be monitored.*

**Reply:** As the referee points out, it is of much more clinical relevance to study the release of growth factor proteins. However, for demonstration purposes we have chosen for the colored mRuby2 here. In a recent paper from Bastings, Koudstaal, et al. (*Adv. Healthcare Mater.* **2014**, *3*, 70–78) where this methodology is used, the release of hepatocyte growth factor and insulin-like growth factor-1 is measured using ELISA detection.

*Minor Concerns:*  
*1. The authors wrote '1 hour' (line no: 165) in one place and '1h' (line no: 173) in the other place. It should be consistent.  
  
2. They assayed the oscillatory rheological and modulus of the formulated hydrogel. In the results section, Standard error has been shown in the graph. It will be good if the authors mention "N", i.e., the number of independent measurements / experiments there.  
  
3. There is typographic error (i.e: Myosatr) in the page of the list of material/equipment.*

**Reply**: We have addressed the minor concerns raised by the referee.

1. We have checked the consistency of the used symbols (track changes)
2. We have added the number of experiments performed in the caption of the figure 1.
3. Typographic errors have been addressed (track changes).

*Additional Comments to Authors:*  
Is it possible to provide some data on growth factors delivery?

**Reply:** See above.  
  
**Reviewer #2:**   
*Manuscript Summary:*   
*It is an interestin new concept with injection into the heart of a biomaterial, which potentially can be mixed with different drugs to improve the stay of the drug in the injected area.  
The description of the production of the biomateriale and its handling in some situations are described clearly.   
It has been injected into the heart with the NOGA method, which also is used in clinical studies.*

*Major Concerns:  
I have no major concern.  
  
Minor Concerns:  
To reach a level where it can be used for clinical therapy, the authors have to look more on the production method, sterility methods etc. However, the present study can be seen as the proof of concept study, which now can be moved into the next research phase for optimization.   
  
Additional Comments to Authors:  
N/A*

**Reply:** We would like to thank the referee for the nice words on our manuscript. We do agree that for the use in clinical therapies, several hurdles have to be taken such as the points raised by the referee. Here, we have indeed chosen to present the concept of the methodology. More details on for instance biocompatibility can be found in the paper from Bastings, Koudstaal, et al. (*Adv. Healthcare Mater.* **2014**, *3*, 70–78).