# Engineering 3D cellularized collagen gels for vascular tissue regeneration

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**Date of reception from the editors:** 09/10/2014

**Date of revision:** 17/12/2014

**General comments**

-The authors would like to take this opportunity to thank the science editor, the Reviewer #1 and the Reviewer #2 for valuable work in improving the overall quality of the manuscript. Authors really appreciated the pertinence of the comments, and the well thought out of the suggestions.

**Editorial comments:**

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (52812\_R2\_RE.docx) is located in your Editorial Manager account. Please download the .docx file and use this updated version for any future revisions.

- Accordingly to the comment, the authors agreed the formatting of the text proposed by the Editor. Moreover, the authors uploaded all the Supplemental Videos to the Editorial Manager with revised captions. Please, note that a new video has been added to the protocol in section 2.4.2, which is related to the harvesting process of the construct. Numbering of the subsequent Supplemental Videos has been changed as consequence.

**Changes made by the Science Editor:**

1. There have been edits made to the manuscript. Please accept (or address) all tracked changes.

- The authors found all the comments and the edits made to the manuscript by the Science Editor very pertinent. Accordingly, the authors addressed all tracked changes.

**Changes to be made by the Author(s):**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

- Accordingly to the comment 1, the authors proofread the manuscript spelling or grammar issues were corrected in red. While reviewing the text, the authors agreed to revise the title too, mainly because the word “fabrication” does not reflect the status of the work. Moreover, the word “regeneration” will allow the manuscript to be referenced and accessible by keyword search much easier then the word “fabrication”.

2. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Currently, there are 3.5 pages of highlighted protocol text. This limit is to ensure that the videography can occur in one day.

- Accordingly to the comment 2, the authors highlighted (2.75 pages, in yellow) all the steps of the protocol that identify the essential steps of the protocol for the video.

3. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

- Accordingly to the comment 3, the authors included all relevant details required to perform the step in the highlighting.

**Reviewers' comments:**

**Reviewer #1:**

*Manuscript Summary:*

This article is absolutely relevant to similar tasks undergone on vascular tissue engineering labs around the world. The description is adequate and the authors provide some detail on potential problems.

*Major Concerns:*

None

*Minor Concerns:*

None

*Additional Comments to Authors:*

N/A

**Reviewer #2:**

*Manuscript Summary:*

The manuscript focus a direct strategy to assemble collagen and smooth muscle cells in a 3D cylindrical geometry useful for the production of vascular tissue and also a way to characterize its mechanical properties in an environment similar to the physiological.

*Major Concerns:*

No major concerns were detected. The manuscript was well organized and well written, and the results attainable by the use of both strategies proposed have very good potential use in the production and characterization of biomaterials.

*Minor Concerns:*

Please find below a list of recommendations to improve the overall quality of the work:

1) In Figure 4, the text showing the brands/models and the screen of the equipment is upside down.

To ensure the validity of the measurement, the longitudinal axis of the construct and the plan of the laser beam have to be perfectly orthogonal to each other. The construct or the laser has to be flipped from the vertical position to the horizontal position. Flipping the construct would require additional equipment in the cell culture hood, which could compromise the sterility of the environment. Therefore, the scanning laser interferometer is flipped and its horizontal position is ensured with a tubular spirit level.  
The authors added additional information in step 2.3.1 accordingly to the previous explanation.

2) Data on glutamine consumption and ammonium formation in addition to glucose consumption and lactate formation would be an excellent addition, given that glutamine is not only an important source of nitrogen to animal cells in culture, but also of carbon and energy. Its consumption results in formation of ammonium, a compound that can be deleterious to cells if accumulated in high proportions. Also, since glutamine metabolism is strongly related to glucose metabolism and that animal cells frequently shown unbalanced (overflow) metabolism in vitro, results of the evolution of concentration of the four compounds mentioned would be more complete regarding the analysis of the metabolic activity of the cells.

The authors would like to thank the Reviewer #2 for pointing out this really important point. The results presented in this work are only representative. Data on glutamine consumption and ammonium formation would certainly improve the overall cell metabolism for further investigation.

3) Still regarding glucose and lactate data, the authors in fact do not report values of rates of consumption and production of these compounds in Figure 10. Variations (deltas) are reported as a function of time, but not the derivatives of concentrations with time (dC/dt), which would indeed represent the rates. Also, are the data shown cumulative? It seems to be the case, given that the culture medium was changed frequently and so, similar values would be expected in equal analysis periods if the metabolism of the cells did not vary greatly in the evaluated intervals. Finally, the caption of Figure 10 describes two figures0 (A and B), but both graphs are shown in the same figure.

Please then correct the discussion and the caption regarding Figure 10.

-According to the comment 3 of Reviewer #2, the authors replaced the graph in figure 10. The new graph now reports the derivatives of concentrations with time (dC/dt), which is more pertinent. In addition, the authors modified the caption as suggested.

4) Please check/correct the following sentences:

- Item # 1.2.1, lines 150-151: Drill a 4.5 mm diameter hole at the center of the ventilated cap of the reservoir tube without damaging filter membrane that covers the aerating holes. Recommendation: insert a THE between "filter" and "membrane

-Item # 2.4.2, lines 283-284: Transfer gently the mature construct from its mandrel to a 100 mm diameter Petri-dish containing 40 ml of fresh medium culture (Figure 5, b and Figure 7, a).

Recommendation: change "medium culture" to "culture medium"

- Item Disclosure: No funding was received organizations or agencies with potential conflict of interests.

Recommendation: change to "No funding was received from organizations or agencies with potential conflict of interests".

- Change "posphate" to "phosphate" in the List of Material/Equipment.

The authors addressed the recommendations suggested by the Reviewer #2 in the comment 4.

5) Please provide the description of the sources/Companies/Catalog Numbers for the following items:

- collagen (despite the description is available in another publication, this compound is too important in the body of the present paper and should be ideally described again)

- pSMCs cells

- HUVECs cells

The authors detailed the extraction process in step 2.1.4 as suggested by the Reviewer #2 in the comment 5. In addition, the authors provided information about collagen and cell sources in the material list (file: 52812\_R1\_102714\_Materials\_Reply.xls).

*Additional Comments to Authors:*

None