Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

Form 2T

ASSESSMENT REPORT OF A PhD THESIS PRIOR TO ITS DEFENSE

(As required by Section 2.1c. of the Regulation concerning doctoral thesis defense at UVa)

Full name: Artur Jorge Araújo Magalhães Ribeiro

Department: Biological Engineering

University or Research Institution: Universidade do Minho, Portugal

Regarding the thesis entitled: Novel Hydrogel-forming Elastine-like Recombinamers For Biomedical Applications

Written by Mr./Mrs.: Arturo Ibáñez Fonseca

Please, report your arguments and critical opinion on the following issues concerning the PhD thesis, writing as much as necessary:

1. Is the topic relevant? Are the research objectives well defined?

The topic is relevant and is in the cutting-edge of Tissue Engineering and Regenerative Medicine (TERM). There is high scientific interest of the research developed throughout the PhD Thesis. The results demonstrate the high impact and interest for the scientific community. The objectives are in accordance with the work flow.

2. Is the selected methodology sound and suitable for the topic and the objectives pursued in the thesis?

The methodology and strategies employed to accomplish the proposed objectives were adequate. The goals of each chapter of the PhD Thesis were achieved using several methodologies. The level of execution of each methodology and objective is high despite the scientific challenge associated with each task.

3. Is the body of reviewed literature up to date and complete? Have all relevant sources been considered and cited?

The reviewed literature is up to date and complete. The sources have been considered and support the need of the work developed.

4. Does the thesis make original contributions that expand the current knowledge on the subject? Are these contributions relevant?

The field of TERM is very demanding and challenging. The results obtained in this PhD Thesis are of great relevance regarding the development of new hydrogels with high potential in tissue engineering as well for the development of a new generation of biosensors. The thesis contributed for a deeper characterization regarding the biocompatibility of ELRs and of hydrogels based on them which can be used in several applications like in bone tissue engineering.

5. Is the thesis structure adequate to explain the research carried out and the results achieved? Is language used properly? Are formal elements, like figures or tables, well laid out and helpful to understand the research and results?

The manuscript is well written and structured. The organization in four chapters, each one corresponding to a published article, make it easy to follow and was very understandable. The level of English is high and the language is appropriated. Figures and tables have a good quality and help to understand the results. Discussion of results is in line with figures and tables.

6. Only if this is a compilation thesis (written in the format of a collection of articles): Is there a clear and coherent connection among the topics and methodology of the different articles that comprise the thesis? Do the introduction and conclusions of the thesis provide a unifying picture of the whole research?

The present thesis despite is a compilation thesis is clear and coherent. The topics are all related with the application of ELRs in TERM specially for the formation, characterization and application of hydrogels. The introduction and conclusions help for the comprehension of the general idea behind the work developed.

7. Please mention three strengths and three weaknesses of this thesis.

Strengths : (1) Quality, relevance and novelty of the work developed; (2) Number of new ELRs developed with a proved application in TERM; (3) Design of a new class of biosensors with great potential
Weaknesses: (1) Figures captions should contain more information; (2) Some extra figures could have been included in Introduction (chapter 1); (3) ———

8. If you think the thesis should NOT be defended in its current form, please mention the changes that you consider MUST be done before it can proceed to defense.

Nothing to declare. The thesis is OK to be defended on its current form.

9. Please mention other changes that MAY be done in order to improve the thesis quality, but that you do not consider strictly necessary to authorize its defense.

Nothing to declare.

10. Any other comments:

It was very easy and scientific challenging to read the manuscript. The goals, initially proposed, were fully accomplished and the final results are very interesting and with high impact. The results could lead to new products with high impact in the field of bone tissue engineering like bone restoration and osteointegration. The combination of two fluorescent proteins with ELRs set this class of biomaterials to be used in several areas like as biosensors.

Please provide your recommendation to the Academic Board of the PhD Program:

- [x] This thesis should be ADMITTED for defense, either in its current form or after taking into account the suggestions made in point 9 of this report.
- [ ] This thesis should be MODIFIED before its admission for defense in order to make the changes requested in point 8 of this report.
- [ ] This thesis should be REJECTED for defense, due to the arguments given in this report.

Place and date: Braga, 08/09/2017

Signature:

Notes: The length of this report is not restricted. Please remember to sign it (digital signatures are accepted).

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

Form 2T

ASSESSMENT REPORT OF A PhD THESIS PRIOR TO ITS DEFENSE

(As required by Section 2.1c. of the Regulation concerning doctoral thesis defense at UVa)

Full name: Antonella Bandiera

Department: Life Sciences

University or Research Institution: University of Trieste

Regarding the thesis entitled: NOVEL HYDROGEL-FORMING ELASTIN-LIKE RECOMBINAMERS FOR BIOMEDICAL APPLICATIONS

Written by Mr./Mrs.: Arturo Ibáñez Fonseca

Please, report your arguments and critical opinion on the following issues concerning the PhD thesis, writing as much as necessary:

1. Is the topic relevant? Are the research objectives well defined?

Yes, the topic is relevant, beloning to the wide field of biomedical biomaterials, regenerative medicine and tissue engineering. The research objectives are clearly stated and described at the beginning of the thesis and at the beginning of each chapter

2. Is the selected methodology sound and suitable for the topic and the objectives pursued in the thesis?

The selected methodology is surely suitable for the topic and the objectives pursued in the thesis, most of it deriving from a well-established experitise of the lab where the work was carried on

3. Is the body of reviewed literature up to date and complete? Have all relevant sources been considered and cited?

Bibliography appears adequate and balanced respect the whole text

4. Does the thesis make original contributions that expand the current knowledge on the subject? Are these contributions relevant?

The thesis lead to a substantial enrichment to the topic of elastin-like based biomaterials and contributes to deepen knowledge in this field

5. Is the thesis structure adequate to explain the research carried out and the results achieved? Is language used properly? Are formal elements, like figures or tables, well laid out and helpful to understand the research and results?

In its complex the thesis results adequate to explain the work that was carried on and the results obtained. Formal elements are generally well integrated with text. Specifically, since the reader is not familiar with the sequences of the recombinamers employed (that are rather complex to remember!), these data could be better evidenced and organized.

6. Only if this is a compilation thesis (written in the format of a collection of articles): Is there a clear and coherent connection among the topics and methodology of the different articles that comprise the thesis? Do the introduction and conclusions of the thesis provide a unifying picture of the whole research?

Yes, in general a fair connection is followed throughout the thesis and a unifying picture of the whole research emerges.

7. Please mention three strengths and three weaknesses of this thesis.

First strenght of the thesis is that the most of its content is already published in high level scientific journals, and this means that quality of the work has been already assessed. Second strenght is that many of the described achievements open the way to further study and development. Third, some not yet published, preliminary results reported have room to be further developed and represent solid bases for further publishable results.

8. If you think the thesis should NOT be defended in its current form, please mention the changes that you consider MUST be done before it can proceed to defense.

Overall the thesis could be presented in its current form, well witnessing all the work done during the doctoral course period

9. Please mention other changes that MAY be done in order to improve the thesis quality, but that you do not consider strictly necessary to authorize its defense.

Please, see above point 7 (weaknesses) and comments in point 10 below

10. Any other comments:

Comments/observations:
- Chapter2: it is true that ELPCG sequence has been already published however, for reader convenience, it would be better to have the sequence and a scheme of that in the thesis text. Possibly, since the reader may be not familiar with the constructs used for the study (that are the key of all the work from the lab!), evidencing both sequences and their schemes at the beginning would make the reading less muddled. For example, one simple

Please provide your recommendation to the Academic Board of the PhD Program:

- [x] This thesis should be ADMITTED for defense, either in its current form or after taking into account the suggestions made in point 9 of this report.
- [ ] This thesis should be MODIFIED before its admission for defense in order to make the changes requested in point 8 of this report.
- [ ] This thesis should be REJECTED for defense, due to the arguments given in this report.

Place and date: 14-Set-2017

Signature: 

Notes: The length of this report is not restricted. Please remember to sign it (digital signatures are accepted).

RESPONSE LETTER TO REVIEWER'S COMMENTS

September 15th, 2017

Dear Prof. Bandiera,

I, Arturo Ibáñez Fonseca, am pleased to answer below your comments regarding the revision of the PhD Thesis entitled "**Novel hydrogel-forming elastin-like recombinamers for biomedical applications**".

Comments:

1. Since many different elastin-like macromolecules (and their related physico-chemical behavior) are employed throughout the study, it is not difficult for the reader to lose the plot and thus maintaining the whole picture is sometime not trivial.

In order to make easier to know the physicochemical properties of the elastin-like macromolecules, a new table has been inserted in the "Resumen" section (**Tabla 3, pages 74-75**), including the bioproduction yield, the theoretical and experimental molecular weight, and the transition temperature (T_t) of the ELRs bioproduced during the thesis (all excepting HRGD6 and $(EI)_2$). This table, in combination with the existing one describing the abbreviated amino acid sequences of all the elastin-like recombinamers (ELRs) used in the Thesis, may facilitate the search of the sequences and the different key ELR properties. Furthermore, the abbreviated amino acid sequences of the ELRs used in Chapter 3 have been included in the Supplementary Information subheading (**Table S6, page 283**, see answer to comment 5), whereas the sequences for the ELRs used in Chapter 2 and Chapter 4 were already available in the corresponding Supporting Information sections.

2. Maybe the structure (likely mandatory) of the thesis doesn't help and force the reader to jump from one section to another, making reading laborious.

As noticed by the reviewer, the structure is imposed by the regulation regarding the presentation of a thesis written in the form of a collection of articles. However, the first section ("Resumen") has intended to create a thread in order to unify the different Chapters of the Thesis.

3. Maybe the final Conclusions and Future directions of the thesis could be better organized starting with Future directions (that reports studies with preliminary results to be completed) and finishing with Conclusions. These last could consist in only one section (without the partition) dealing with the overall significance of the work exposed, rather than a point-to-point resume of the 4 chapter already present in each chapter.

The Conclusions section has been changed as suggested by the reviewer in terms of order. In this way, the subheading called "Future directions" has been placed before the "Conclusions" one **(pages 333-350)**. However, I think (in agreement with my supervisors) that the point-to-point summary made as a conclusion of the Thesis is the finest way to describe concisely the key findings showed in each chapter.

4. Chapter 2: it is true that ELPCG sequence has been already published however, for reader convenience, it would be better to have the sequence and a scheme of that in the thesis text. Possibly, since the reader may be not familiar with the constructs used for the study (that are the key of all the work from the lab!), evidencing both sequences and their schemes at the beginning would make the reading less muddled. For example, one simple solution may be to match the list of sequences present in

table 2, pg. 51 in the Spanish version with the respective schemes (by the way, in this table the sequence of ELPCG seems not present).

It has been specified that the ELR named HRGD6 in Tabla 2 of the "Resumen" section is the one used in the formation of ELR-CFCGs (**page 51**), which I suppose is the type of hydrogel that the reviewer refers to. Furthermore, the sequence of this ELR has been also included in the Supporting Information subheading of Chapter 2 (**page 231**). In addition, the schemes of all the ELRs have been included in a new figure in the "Resumen" section (**Figura 1, page 53**)

5. Chapter 3: in the constructs used, ELR-E-BMP-2 and ELR-E-RGD it is stated that the sequence that is sensitive to elastase in the constructs is the (VGVAPG)3 motif. On the other hand, it is less clear in the text, which is the non-sensitive ELR used as control. Nor it is explained in Fig. 2d and the related text (pg. 243-244). From "tabla 2" pg.51, it seems to be $(EI)_2$ (ELR no sensible a elastasa), however, intriguingly, the sequence reported on pg. 52 for this construct has the same (VGVAPG)3 motif as well. May be is the reported sequence wrong? (I had not access to ref. 44 indicated for this construct to check it).

In order to address the reviewer's comments, we have cited the $(EI)_2$ sequence in Tabla 2 of the "Resumen" in the corresponding section of Chapter 3, both in the text (**page 249**) and in the Fig. 2d caption (**page 250**). Furthermore, the sequences of all the ELRs used in Chapter 3 have been included in the Supplementary Information section of this chapter (**Table S6, page 283**), which has been also referenced in the text, in the same way than Tabla 2 of the "Resumen" section.

On the other hand, the reviewer is right about the inaccuracy of the $(EI)_2$ sequence reported in Tabla 2 of the "Resumen" section in **page 52**, since the $(VGVAPG)_3$ motif is

not present in the ELR named "$(EI)_2$ (ELR no sensible a elastasa)". Therefore, this domain has been removed from the $(EI)_2$ sequence in the aforementioned table and in the newly created Table S6 in the Supplementary Information section of Chapter 3 (**page 283**).

6. Chapter 4: it is stated that "a great amount of molecules is present in a SELR-FP-based hydrogel, so it will be clearly visible by in vivo imaging systems or other instruments dedicated to detect fluorescence." (pg 294). However, being the two FPs from a heterologous organism (Aequorea coerulescens) adverse reactions could be expected for the in vivo tracking use of these constructs despite the good biocompatibility of the ELR backbone shown in Chapter 2.

The reviewer makes a very good appraisal regarding the possible *in vivo* adverse reactions when injecting a fluorescent hydrogel containing heterologous proteins, such as the fluorescent ones described in Chapter 4. Actually, this is going to be one of the next steps towards determining the safety and the feasible application of SELR-FP-based fluorescent hydrogels in biomedical applications. In this regard, we are planning future *in vivo* experiments which will involve the evaluation of the biocompatibility by the methods described in Chapter 2, combined with qPCR assays for the assessment of the expression of different cytokines and other markers of inflammation.

Thank you for reviewing this PhD Thesis.

Sincerely,

Mr. Arturo Ibáñez Fonseca
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