

September 6, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Griffin et al 12789-edited).



Title: Control of Stem Cell Fate by Engineering their Micro and Nanoenvironment

Author: Griffin Michelle F, Butler Peter E, Seifalian Alexander M, Kalaskar Deepak M

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 2429

The manuscript has been improved according to the suggestions of reviewers:

Responses to Reviewer 1.

- a) **The authors should provide justification for the need of an additional review on the topic or better highlight in what consists the originality of their manuscript**
We have provided justification on page 5 last paragraph.
- b) **The authors and/or the Editor should ask permission for reproduction of these data to whom it may concern.**
We now have permission for all figures.
- c) **Page 4 line 2 «therapeutic» , could it be substituted by regenerative**
We have substituted this.
- d) **Page 4 line 8 «ability to maintained for» , ???**
We have changed the word to expand.
- e) **Page 4 line 21 etc. «In their in vitro environment stem cell fate is determined by a reservoir of biochemical and biophysical clues [6]. The instructions from their stem cell niche will govern their niche environment, controlling » some words can be eliminated.**
We have now eliminated some words.
- f) **Page 5 «The physical environment has shown to be an alternative means of controlling cell fate[10] » the phrase can be eliminatedpage 5 «Biochemical clues in the form of growth factors and cytokines have also shown to provide important instructions to stem cells in vitro[15]. » the phrase can be eliminated, redundant with refs 7-9**
This phrase has now been eliminated.

- g) **Page 5 «T(a) topographical, (b) chemical and (c) molecular clues » I guess the authors ment «molecular genetic», otherwise it is not clear which the difference is with chemical**

We have now changed the word as per reviewers suggestion.

- h) **Page 10 «The hMSCs adopted the morphology of the differentiated cells. » the phrase can be eliminated.**

This Phrase has been eliminated.

- i) **Page 11 line 15 «cue» , could it be substituted by «clue» for being consistent**

This has now been changed to clue.

- j) **Page 11 line 2, check the plural «molecules»**

The word has been changed to molecules.

- k) **Page 13-14 the work by Coletti D et al. in J Biomed Mater Res A. 2009**

Nov;91(2):370-7. doi: 10.1002/jbm.a.32243.seems very relevant for SAM and could be worth citing it

Paper suggested by reviewer is indeed very interesting, however work presented in this study look at the effect of Cysteamine attached via SAMS on differentiation of C2C12 cells. It does not look at direct effect of SAMS. Authors believe although this paper is very interesting, does not fit with current work which exclusively talk about effect of SAMS (-CH₃), hydroxyl (OH), carboxyl (-COOH), amino (-NH₂) and thiols (-SH) on differentiation of the stem cells.

- l) **Page 14 botttom «transfection efficiency» should be «applicability» or similar, usually viral infection is quite efficient**

This word is correct and should be transfection efficiency.

- m) **Page 14 bottom «Adenoviruses have been illustrated to have higher transfection efficiency but their use is less discouraging in vivo responses including early failures has led » , ??? please rephrase**

We have now rephrased this.

- n) **Page 15 «Non-viral gene delivery is the most commonly used in the form of a DNA plasmid, which is usually extracted from bacteria[105]. » the phrase can be eliminated**

The phrase has been eliminated.

- o) **Page 16 «To provide an overview, siRNAs are 19-22 base pair nucleotides which when delivered into the target cell will cause the specific knockdown of the gene that is complimentary to its sequence. The siRNA causes silencing of the specific gene by unwinding of the double strand in the RNA-induced silencing complex (RISC), followed by recognition and cleavage of the complementary strand of endogenous mRNA105-108]. Several areas of research have endeavoured to use siRNA to induce specific differentiation of stem cells. » the paragraph can be**

eliminated and the notion referred to by the citations, for those who want to have the basics of siRNA.

This paragraph has now been eliminated.

- p) Page 17 I guess the symbol TM should be added to «Oligofectamine and siPORT»**

This symbol has been added.

- q) Page 18 «Nanotopographies hold great promise due to the ability to sustain their effect in vitro better than chemical modifications of biomaterials, as they are not affected by degradation. » the authors should further explain or provide bibliographic support for this statement**

Following sentence has been added to support statement 'Using nanotopographies of biomaterials to guide stem cell fate holds great promise, as surface modifications are not affected by degradation over time unlike soluble growth factors '.

Responses to Reviewer 2.

- a) The authors included 4 figures derived from other investigators published papers without any specific comments regarding the copyright. The authors have to check this issue.**

We now have permission for all figures.

- b) The authors have to be consistent in abbreviation. For example, adult stem cells were described as ASCs, ADSs, or ADSCs.**

We are now consistent in the abbreviations for example ASCs is now adult stem cells throughout the manuscript.

- c) The authors described several papers related with surface topography and chemicals in the text. However, the same contents were omitted in the Table 1 and 2. You had better include the facts and references in the tables.**

We have modified both the table and included the references.

Responses to Reviewer 3

- a) A thorough review of the punctuation is strongly advised (especially the use of comma).**

This has now been done.

- b) Examples: Page 2: "Stem cells are capable of long-term self-renewal and differentiation into specialised cell types, making them an ideal candidate..." - a comma should be added between types and making**

This has now been added.

- c) **"The control of stem cell fate has become a major area of interest, in the field of regenerative..."** – this is the opposite case: the comma after interest should be removed
This comma has now been removed.
- d) **"...delivered via biomaterials to be able to provide clues to determine stem cell differentiation"** – rephrase the sentence, in order to avoid or reduce the repetition of "to" **"This review aims to provide an overview of the topographical, chemical and molecular clues that biomaterials can provide to guide stem cell fate."** – rephrasing is advised so as to avoid the repetition of "provide"
This has now been rephrased.
- e) **An alternative approach is to provide genetic clues including delivering DNA plasmids and small interfering RNAs (siRNA) via scaffolds has also shown to influence stem cell fate."** – rephrase to clarify
This has now been rephrased' An exciting area of research is non-viral gene delivery via biomaterials to direct stem cell function'.
- f) **Page 4: "The second type of stem cells, which has attracted extensive research interest are adult stem cells"** - replace are with is represented by, since the grammatical concordance requires it (the subject is "type", the predicate cannot be "are")
This has now been replaced.
- g) **"The extracellular matrix in the in vitro stem cell niche is arranged into complex topographic features..."** – Italics for "in vitro" Page 10
This is now in italics.
- h) **"However, it is thought, that the elasticity of the ECM induces changes in the focal adhesion protein activity and remodeling"** - remove comma after thought
The comma has now been removed.
- i) **Page 11: "...is provided by the ECM, which regulate a key number of cellular functions..."** – replace regulate with regulates, since ECM stands for extracellular matrix
This has been replaced.
- j) **Page 13: "After functionlisation the gels were able to direct the differentiation..."** – add a comma after functionlisation Page 14:
The comma has now been added.
- k) **"With an emergence of plasma surface modification studies over the last five years which can easily modify the chemical properties of the surfaces and coat the surfaces with different functional groups, the use of chemical groups controlling stem cell fate is likely to receive extensive research due to clear evidence that surface chemistry clearly influences stem cell behavior and their**

differentiation.” – sentence needs rephrasing, since it is too long and the meaning of some parts become somewhat blurred

This has now been rephrased.

l) Page 18: “Future prospectives” vs. “Future perspectives”?

This has now been changed.

m) We also recommend the authors to provide the copyright permission for all figures that are included in the manuscript.

We have now obtained copyright permission for all figures.

Responses to Reviewer 4.

No changes required.

We would like to thanks all the reviewers and editor for their valuable input to improve this manuscript.

Sincerely yours,

Dr Deepak Kalaskar