Novemvber 26, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6253-review.doc).

Title: Sonoporation: Gene transfer using ultrasound

Author: Minoru Tomizawa, Fuminobu Shinozak, Yasufumi Motoyosh, Takao Sugiyam, Shigenori Yamamoto, Makoto Sueishi

Name of Journal: World Journal of Methodology

ESPS Manuscript NO: 6253

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

Reviewer 1
I would recommend to provide more data on in vitro or in vivo efficency comparison with the routenly used methods

Response: Sonoporation with microbubbles enhances gene transfer with liposome in vitro and in vivo. Gene transfer of siRNA with Lipofectamin 2000 is enhanced with sonoporation. This part was added in the end of microbubbles.

Reviewer 2
Gene transduction method using microbubble and ultrasound is referred to as sonoporation. Sonoporation is an interesting technique of gene delivery. Since the description of transdermal delivery of insulin by ultrasound, various research groups have tested the technique in various fields from embryogenesis to arthritis. The review entitled "Sonoporation: Gene transfer using ultrasound" submitted by Tomizawa et al is a comprehensive account of several transfection procedures applied in vivo and in vitro as well. The authors have compared different gene delivery procedures with sonoporation.

This method of gene transduction appeared to be safe. The review is appropriate for general readers as well as researchers who are interested in new techniques.

However the authors should include more information (e.g., characteristics that is required to be an effective contrast agent) regarding contrast agent or microbubble material as it is referred to in the text.

Reseponse: Positive charge is superior to neutral because plasmids are negatively charged. This part was added at the end of microbubbles.

The review will be incomplete if it does not cover the applications of the technique beyond cancer and arthritis.

Response: Tissue engineering is the other aim of sonoporation. This part was added in the second line of introduction.

Reviewer 3

The manuscript is a review article about the use of sonoporation for gene transfer. It is a relatively new technique, so a review article discussion is a worthwhile topic. The article tries to highlight the importance of sonoporation, but its unique advantages or important niche applications are not depicted well. In general, the article simply repeats finding from the literature in a confusing manner, and does not synthesize to make a unique argument/contribution in a coherent way. Major revisions must make it clear what argument you are trying to make in each section, written in a clear manner.

Do all of the literature suggest positive results? Point out conflicting or complicating findings and make your overall opinion/assessment.

Response: Limitations of sonoporation are low gene transfer efficiency and apoptosis. This part was added in a new section as “e. Limitations” in “Sonoporation”.

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1. The core tip is poorly written and needs editing for English.

Response: The core tip was changed.

2. The first sentences of the abstract and the introduction are too redundant. Rephrase the wording in the different sections.

Response: The first sentence of the abstract was deleted. The first sentence of introduction was changed.

3. Intro, There is a poor understanding of viral gene transfer. Viral capsids are not produced by recombinant vectors. Adeno-associated vectors do not cause a major immune response like adenovirus does. Sonoporation has been used with AAV and this work should be mentioned. For example: Ultrasound targeted microbubble destruction enhances gene transduction of adeno-associated virus in a less-permissive cell type, NIH/3T3. Jin L, Li F, Wang H, Li Y, Wei F, Du L. Mol Med Rep. 2013 Aug;8(2):320-6. Ultrasound targeted microbubble destruction stimulates cellular endocytosis in facilitation ofadeno-associated virus delivery. Jin LF, Li F, Wang HP, Wei F, Qin P, Du LF. Int J Mol Sci. 2013 May 7;14(5):9737-50. Elucidating the mechanisms behind sonoporation with adeno-associated virus-loaded microbubbles. Geers B, Lentacker I, Alonso A, Sanders NN, Demeester J, Meairs S, De Smedt SC. Mol Pharm. 2011 Dec 5;8(6):2244-51.

Response: This part was added in introduction.

4. Gene Therapy section has very redundant portions from earlier sections like …plasmid DNA rarely integrates…redundant portions should be deleted. The listing of clinical trials is not important because they have been extensively reviewed in other articles. You should only focus on the work with sonoporation and only mention the clinical trials that have shown promising therapeutic results, not all the ones that have been attempted which is not that important and has been covered elsewhere. The section about frizzled-9 is confusing, what point are you trying to make?

Response: Gene Therapy section was deleted.

5. Discussion of gene gun goes nowhere. Has the strategy been successful? Point out that the gene gun does not work so thus sonoporation should be attempted.

Response: Gene gun section was deleted.

6. Discussion of electroporation and lipofection is unfocused. Make main idea sentences to make your key point in each section. the cystic fibrosis and the 112 trials are not worth mentioning because they do not make any coherent point. Why are you mentioning it? what is the key point?

Response: Electroporation is the only method of gene transfer to primary cell. However, it requires surgical procedure and high voltage. Lipofection is popular, but its gene transfer efficiency is not satisfactory. These weak points will be expected to be solved by sonoporation. These points were added in electroporation and lipofection. Clinical trial with lipofection was deleted.

7. Sonoporation does not sound safe if it causes cell death (section d).

Response: "safe" was deleted in section "d. sonoporation".

8. In vitro uses of sonoporation are largely irrelevant because there are good methods available for in vitro gene transfer.

Response: In vitro use of sonoporation is a model of in vivo sonoporation. This part was added in the beginning of the second paragraph of "a. emergence of sonoporation".

9. The mechanism of enhancement by the contrast agents is not explained clearly.

Response: Contrast agents are the same as microbubbles. "Contrast agents are shells containing gas. Ultrasound scatters on the surface of contrast agents, and are visible as high echo on the display of diagnostic ultrasound. Physical and biological characteristics of contrast agents are basically the same as those of microbubbles." was added in "c.

microbubbles".

10. …when the irradiated cells underwent apoptosis…explain why this is a good thing.

Response: This part was moved to a new section "e. limitations" and discussed.

11. …microbubbles are unstable…this sounds like a serious disadvantage to the method.

Respone: This sentence was changed to "It is difficult to modify surfaces of microbubbles with functional molecules for targeting."

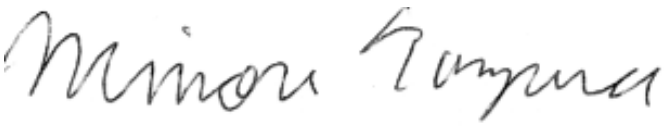
12. The summary is overstated. Sonoporation may offer some specific advantages which should be mentioned. it is too early to say it might become a viable option for gene therapy.

Response: Summary was changed.

13. Fig. 1 is very simple. The first two sentences of the legend are too redundant. Both legends could be explained better. How do you know free radicals are formed in Fig. 2?

Response: Figure 1 legend was changed to "Nucleic acid such as plasmids enters the cells through the membrane pores that are formed with ultrasound." "Free radicals" in Figure 2 was deleted.

Sincerely yours,



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