Reviewer’s report

Title: Effects of pamidronate disodium on the loss of osteoarthritic subchondral bone and the expressions of cartilaginous and subchondral OPG and RANKL in rabbits

Version: 3 Date: 11 August 2014

Reviewer: Giuseppe Musumeci

Reviewer’s report:

General comment
First of all please revise the punctuation and space between the words throughout the text and add the appropriate abbreviations in all text (the PAM meaning must be specified in the introduction and not only in the abstract). Please re-read the text carefully, there are some careless mistakes on the English language.

Please strengthen better the aim of your study in the abstract and in the background section.

Please add also the MMP-9 and TLR-4 in the aim and in the conclusion of the study. It seems that these results were added later and weren’t present in all text.

Abstract
Please reformulate better the abstract adding the suggestion above.

Please reformulate the following sentence of the results section with the correct verbs: “Micro-CT, Safranin O and rapid green staining analyses indicated that PAM treatment for two or ten weeks could completely prevented or reversed osteoarthritic subchondral bone loss and cartilage surface erosion.. “

Background
Please reformulate this section, the description of OPG and RANKL must be improved and updated. The authors should deepen different recent and relevant aspects of these molecules correlate with OA. I recommend seeing the following recent and interesting suggested paper and commenting them: (e.g. “In rat with glucocorticoid-induced osteoporosis, RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training). Histol Histopathol. 2013;28:1185-1196”. – “The effects of physical activity on apoptosis and lubricin expression in articular cartilage in rats with glucocorticoid-induced osteoporosis. J Bone Miner Metab. 2013 May;31(3):274-84”.

Also please add some information about pamidronate disodium (PAM), other bisphosphonates, MMP-9 and TLR-4 protein.

Materials and Methods
Please specify the manufactures used for antibodies, kits, equipment and other
chemicals with their correct addresses in all materials and method section.

Animals and study design section
Line 10, please replace fixed.
Line 14, please explain better the ACLT technique.
Line 18, please correct the font size.

Histology and OARSI score section
Lines 18, 19 please fix the symbols.
Line 20, please add cartilage samples.

Immunohistochemical localization of OPG and RANKL section
Line 27 please add cartilage and subchondral bone sections.
Please add the positive control.

Why did the authors not analyze also MMP-9 and TLR 4 protein in both cartilage and subchondral bone? Please explain it or add these results to strengthen the findings to better readers understanding. Please add the relative results and figures about these two proteins.

Discussion
Page 12, line 27 the word “osteoporosis” is in different font, please fix it.
The discussion is too long and boring, please shorten this section. Moreover, in the conclusion add the results finding about MMP-9 and TLR 4 protein in both cartilage and subchondral bone. Clinical relevance of your work and some important suggestions for the scientific community must be added.

Figure legends
Please reformulate and add more details to all figure legends.
Please use capital letters to explain each image in figures 1 and 3.
Please revise the symbols in figures 5a and 5b.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests