Janelee Li

Assistant Editor

Cosmetics

Dear Janelee Li,

I would like to thank you and the reviewers for helping us improve our manuscript entitled “Facial Skin Lifting and Brightening following Sleep on Copper Oxide Containing Pillowcases”.

We have carefully revised the manuscript according to the Reviewers comments. The revisions in the manuscript are highlighted in red. Below are the responses, point by point, to the Reviewer comments. For ease, the Reviewer comments are in black and our responses are in blue.

We hope very much that you will now find the manuscript appropriate for publication in Cosmetics.

Sincerely,

Dr. Gadi Borkow and Dr. Adriana del Carmen Elías

Reviewer 3:

The article “Facial Skin Lifting and Brightening following Sleep 2 on Copper Oxide Containing Pillowcases” demonstrated the beneficial effects of copper oxide particles on the skin.

I found the manuscript very interesting, it is well written and the data presented are exhaustive and in agreement with the conclusions that the authors presented. The technology presented was also innovative and intriguing.

Although one important issue was not addressed at all by the authors, which was the proved cytotoxicity of copper oxide particles on the skin. Recent papers clearly demonstrated that copper particles, above a certain concentration, may have harmful effect on skin cells, both fibroblasts and keratinocytes. They may also activate apoptotic mechanisms and cause DNA damages (Luo et al, 2014, Int J Nanomed; Alarifi et al 2013, Int J toxico).

In this article only the beneficial effect of Cu was described but the potential toxicity of the Cu particles never mentioned, especially if Cu was used above a certain concentration.

Could the authors address this point? Do the authors have any idea on the amount of copper particles that is delivered on the skin by the pillow cases? Could they talk about the limit of their technology in order to keep under control the safety issue? Please add this info in the introduction and/or discussion part.

The safety of using the pillowcases is obviously a very important issue. It should be noted that the copper oxide particles embedded in the studied pillowcases are not nanoparticles. Their size is at least 10 times the size of the nanoparticles studied in the two manuscripts mentioned above (Luo et al, 2014, Int J Nanomed; Alarifi et al 2013, Int J toxicol). Nanoparticles below 100 nm of size demonstrate significant more cytotoxicity than particles that are above 100 nm. In our case, the size of the particles ranges between 500 to 2000 nm. Be as it may, also copper oxide particles may be cytotoxic, as cells in cell culture may be more susceptible to the environment than when in tissue such as when being part of the skin. Copper oxide is basically non-soluble, and the amount of copper ions that are liberated into the skin moisture are ppm (less than 0.5 nM), concentrations that do not cause cytotoxicity, as we reported previously (Philips et al. Beneficial regulation of fibrillar collagens, heat shock protein-47, elastin fiber components, transforming growth factor-beta1, vascular endothelial growth factor and oxidative stress effects by copper in dermal fibroblasts. Connect Tissue Res 2012, 53, 373-378). No apoptosis, DNA damage, or any atypia was noted. On the contrary, the presence of copper oxide at 0.5 nM, in addition to stimulating the production of extracellular skin proteins (as discussed in the Discussion section), importantly confer protection to the dermal cells from oxidative stress. However, this was demonstrated in cell culture and not in whole skin tissue or skin biopsies, and thus mentioning this in the manuscript seems to us misleading.

As mentioned in the Results section, no adverse reactions were noted in any of the study participants. Similarly, in 13 previous clinical studies with different textiles as well as pillowcases impregnated with the copper oxide particles not a single adverse reaction was recorded. Thousands of consumer products containing copper oxide particles, including pillowcases, are being sold yearly, with no reports of any adverse effects. Medical textiles that contain copper oxide particles are in use more than 3 years now, with more than 150,000 patient days, with not even one adverse reaction recorded. The safety of the copper oxide impregnated products was mentioned in the discussion section in the following paragraph to which the underlined parts were now added (lines 224-232): “Also, in similarity to previous thirteen studies with pillowcases, hospital linens and other consumer products containing copper oxide [30,31,35,36,44,45], the use of copper oxide containing pillowcases did not cause skin irritation, itching, or any other adverse reactions. The amount of copper ions released to the skin moisture is in the ppm range (unpublished data). Copper oxide impregnated products such as socks, apparel, adult diapers, and hospital linens, are in use worldwide for several years, with no reports of adverse reactions. This is in accordance with the very low risk of adverse reactions due to dermal copper contact [46]. Copper oxide is found in multivitamin pills and dietary supplements since copper is an essential mineral and an oral daily consumption of 1-2 mg is recommended [14].”