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Rapid *in-situ* Subcutaneous Bacteria-Killing through Red Light Inspired Reactive Oxygen Species from Graphene Oxide/Ag Nanoparticles Wrapped with Collagen Film

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Introduction: Despite strict sterilization measures, infections caused by artificial implants have not been eliminated. As a broad-spectrum antimicrobial agent, silver nanoparticles (AgNPs) with rapid and highly efficient synergistic bacteria killing of AgNPs, which is achieved by inspiring AgNPs' strong photocatalytic capability using visible light together with the innate antimicrobial ability of Ag^+ [1] have been incorporated into coatings to enhance the antibacterial performance [2,3]. GO is conducive to the distribution of nano-silver and strengthen the photodynamic antibacterial ability [4]. Collagen can improve the biocompatibility of biomaterials, reduce the cytotoxicity of silver ions [5].

Materials and Methods: PDA was applied to introduce $–NH_2$ to combine the AgNPs and GO composite onto titanium surface. The physical and chemical characteristics of the PDA/GO/AgNPs/Col-Ti were studied by various techniques, including transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), the electron spin resonance (ESR) spectroscopy and scanning electron microscopy (SEM). *In vitro* responses of both bacterial and mouse calvarial cells were evaluated by using S. aureus and E. coli and mouse calvarial cells line MC3T3-E1. The *in vivo* responses of subcutaneous tissues were revealed by subcutaneous inflammatory response in mouses.

Results and Discussion: The results demonstrated that the GO/AgNPs/Col coating have fast and efficient photodynamic antibacterial and sustained effective physical antibacterial (Figure 1). And the in vivo experiment show the coating also has excellent antibacterial ability in the body, and its biocompatibility is very good, the cells can be very good growth and reproduction (Figure 2).

Conclusion: This work provides a novel route to endow the implant materials with highly effective self-antibacterial activity as well as good biocompatibility through combining the physical bacteria-killing of AgNPs with their photodynamic action inspired by 660 nm visible light with some penetrating capacity, thus achieving the rapid in-situ disinfection of subcutaneous implants.

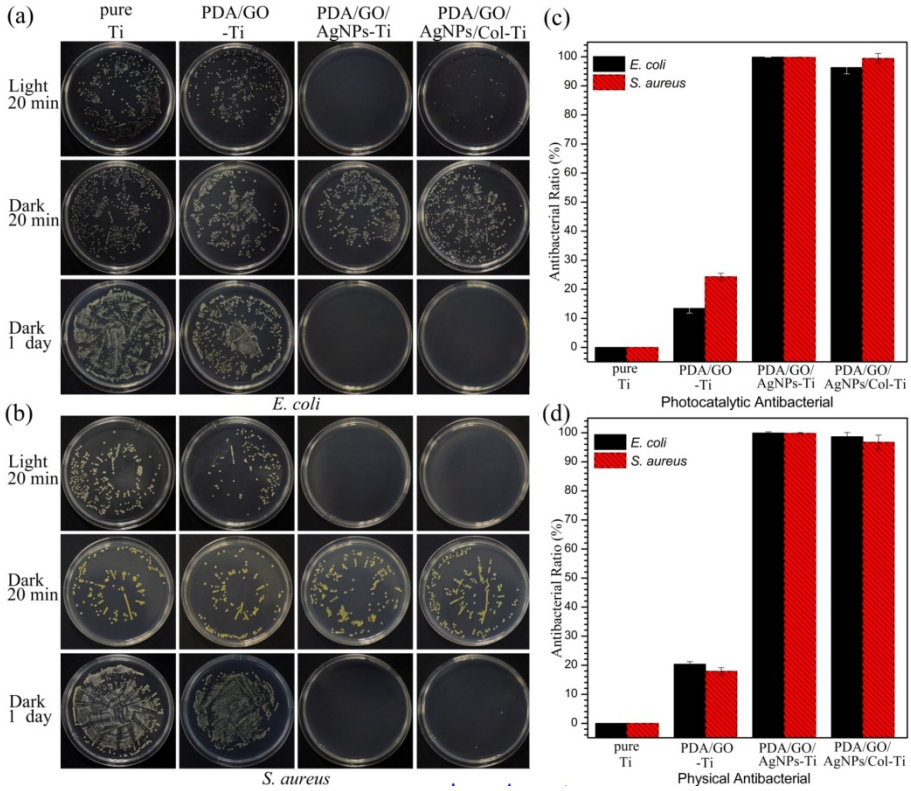

Figure 1 The surface plate of the samples with (a) *S. aureus*, and (b) *E. coli* culturing with 660 nm light irradiation for 20 minutes, culturing for 20 minutes in the dark, or culturing for 1 day in the dark. (c) photodynamic antibacterial inspired by 660 nm light against *S. aureus* and *E. coli*, and (d) physical antimicrobial against *S. aureus* and *E. coli*.

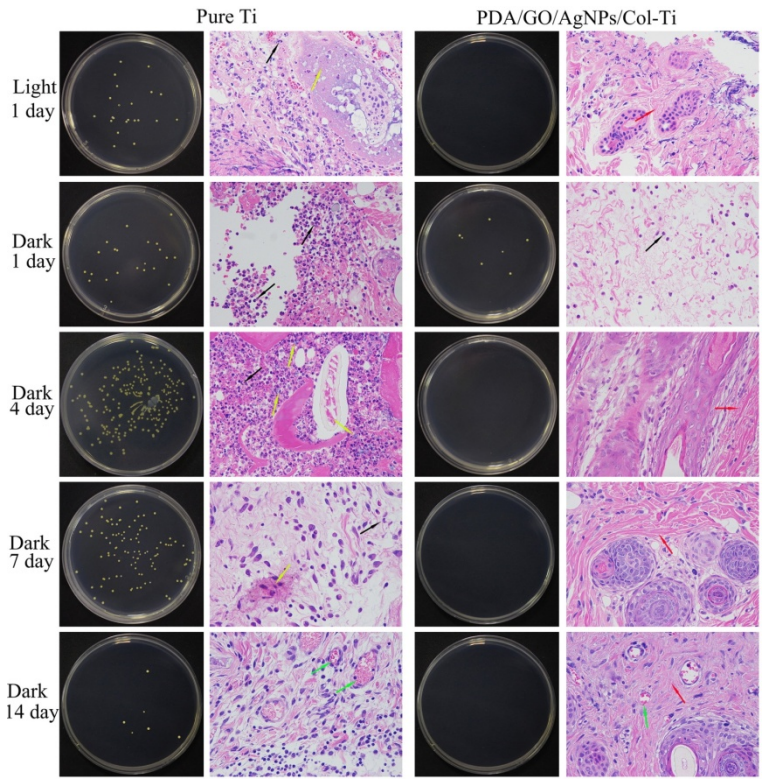

Figure 2 *In vivo* assay. The surface plate was made by take tissue fluid from the wounds , and the histological section was made by the tissue contacted with the samples. the neutrophils inflammatory cells were indicated by black arrows, yellow arrows showed the tissue necrosis cells, red arrows indicated relatively orderliness connective tissues, and green arrows indicated newly formed tissues.

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