Antimicrobial activity of graphene nanoplatelets against *Staphylococcus aureus*.

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The control of bacterial growth is one of the most challenging environmental issues, since surfaces exposed to media rich in microorganisms can be damaged or lose their functionality.

In this work, we have investigated the antimicrobial properties of Graphene Nanoplatelets (GNPs) in acqueous suspensions against *Staphylococcus aureus* to demonstrate the possibility to use graphene based material in environmental applications. In order to study the GNPs toxicity and antimicrobial activity in an in vivo model, we report an antimicrobial study of graphite nanoplatelets on the *S. aureus*-*Caenorhabditis elegans* infection model.

If compared with the traditional antimicrobial agents, nanomaterials can go beyond some classical limitations, such as antibiotic-resistance bacteria development and release of highly toxic metals or biocides. In this context, graphene based materials are emerging as promising antimicrobial agents [1]. The exact mechanism of bactericidal effect it is still not well known, and it is strongly dependent on their surface chemistry. In GNPs produced from graphite intercalation compound (GIC), the mechanical wrapping, puncturing and damaging of the cellular membrane are the only observed mechanisms, without any production of reactive oxygen species (ROS) [2]. Conroy et al. have shown that non-oxidized nanoplatelets typically do not produce ROS, demonstrating their high biosafety, thus enabling their use as antimicrobial agents [3].

In the present work we have produced GNP using GIC as a precursor. In the first step of the process, GIC powders are thermally expanded in order to obtain expanded graphite. We have investigated two different expansion conditions in order to produce nanoplatelets with different lateral size distributions and thicknesses, with the scope of assessing how the morphology of GNPs can affect their antimicrobial properties [4]. The expanded graphite is then exfoliated by liquid-phase exfoliation in a biocompatible solvent.

References

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The antimicrobial tests have been performed on the *S. aureus* cells with the as prepared suspensions. About 5 x 10^7 cells/ml were incubated in PBS at 37°C with GNPs at different concentrations ranging from 10 to 750 μg/ml under shaking for 24h. The viability of the bacteria cells was evaluated by the Colony Forming Units (CFU) method. Although GNP produced with GIC expanded at different conditions (1150°C for 5s and 1050°C for 30s) resulted in different lateral size dimensions (AFM and SEM data not shown), the concentration dependent antimicrobial activity showed no remarkable size-dependent antimicrobial effect in the studied range (Fig.1e). Nevertheless, at high concentrations (i.e. 750 μg/ml) the agglomeration effect of the $GNP_{1150°C}$ resulted in a drop of the antimicrobial effect.

As shown in Fig.1 (c,d), the mechanical antimicrobial effect is intrinsically enhanced when a single GNP interact with cells, so it is strongly dependent by the suspension stability quality.

In order to study the antimicrobial properties of a stable suspension, a mixture of water and Pluronic F108 surfactant has been used as medium in the liquid exfoliation phase. The obtained stable suspension lasted more than 3 months without any sedimentation. Fig.1f shows the antimicrobial effect of the so produced suspensions, in a three weeks shelf-life experiment.

As a final test, using the nematode *C. elegans* as animal model, we found the ability of GNPs to reduce *in vivo* the infection in this mini-host model (Fig.1g), demonstrating the possibility to use GNPs as an antimicrobial and biocompatible material in environmental applications.

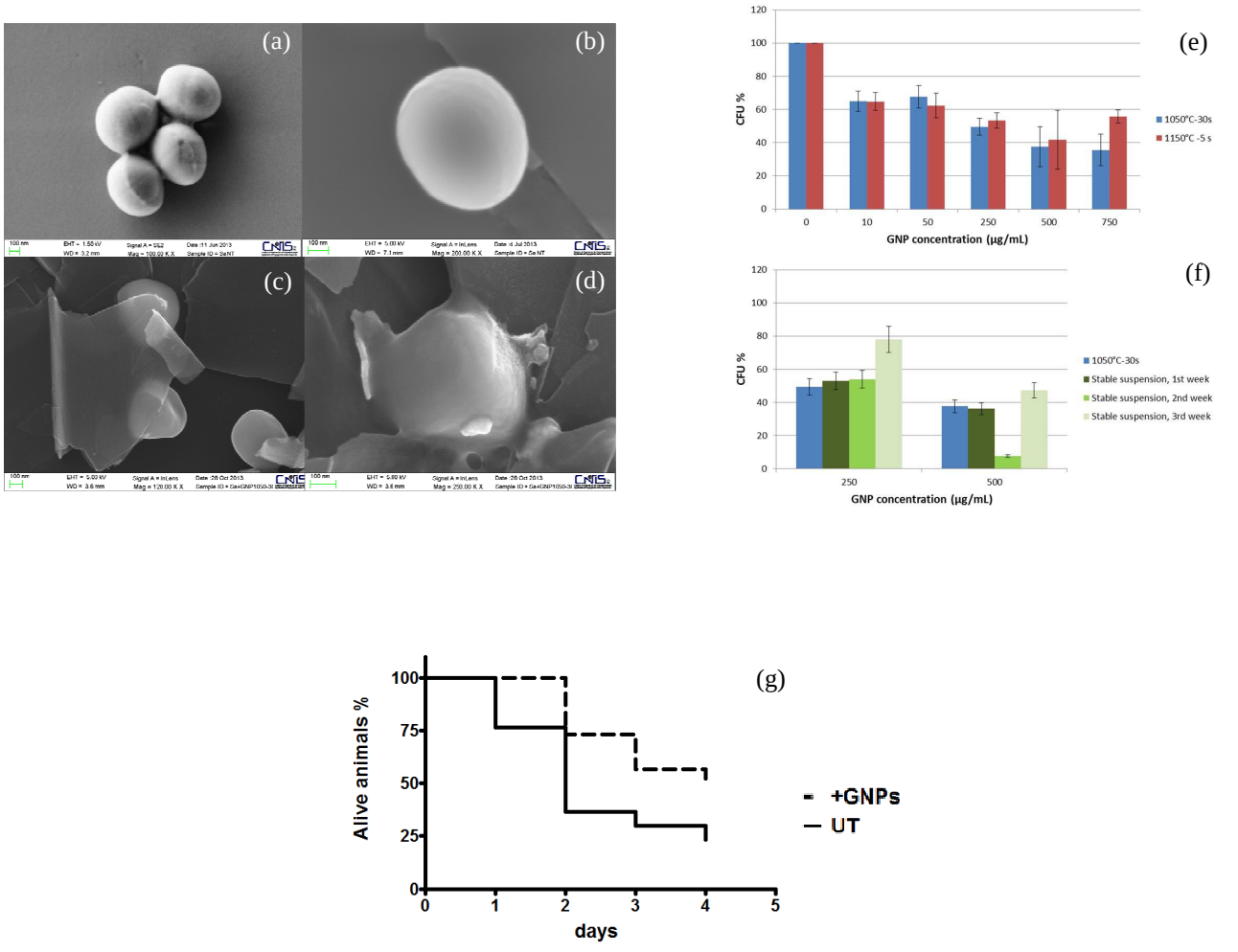

Figure 1: (a,b,c,d) SEM Images of treated and untreated *S. aureus* cells. (a,b) Untreated cells at different magnitudes. (c,d) treated *S. aureus* cells. Fig. 1(d) shows the effect of wrapping, typical of a suspension treatment. (e) Antimicrobial effect of two different GNPs at different concentrations. Loss of cell viability rates was obtained by colony counting method. Error bars represent the standard deviation. (f) Antimicrobial effect of the same GNPs suspension as a function of the first three weeks of shelf-life at two different concentrations. (g) *In vivo* antimicrobial test results on *S.aureus-C.elegans* infection model.