# Black phosphorus-incorporated hybrid nanomaterials as a theragenerative platform for bone tissue engineering

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## INTRODUCTION

As a minimally invasive therapeutic approach, phototherapy is an effective substitute for traditional cancer therapies. Black phosphorus nanosheets (BPNs) with excellent photothermal effect has recently drawn a considerable attention in bone cancer therapy. However, there are some bottlenecks revolving around BPNs—being highly prone to oxidation and a high aggregation tendency in the presence of salts[1].

Bioactive glass (BG)-based sol-gel derived hybrid materials are of particular interest in bone tissue regeneration. As calcium is vital for BG's bioactivity and osteoblasts activities, its incorporation into the BG hybrids is still problematic[2].

Our group recently shed light on the selective anticancer potential of 2D BPNs against SAOS-2 W/WO laser irradiation through ROS production (Fig. 1)[3].

This study is a continuation of our previous work aiming at developing an anti cancer regenerative hybrid biocomposite for bone cancer therapy and regeneration (Fig. 2).

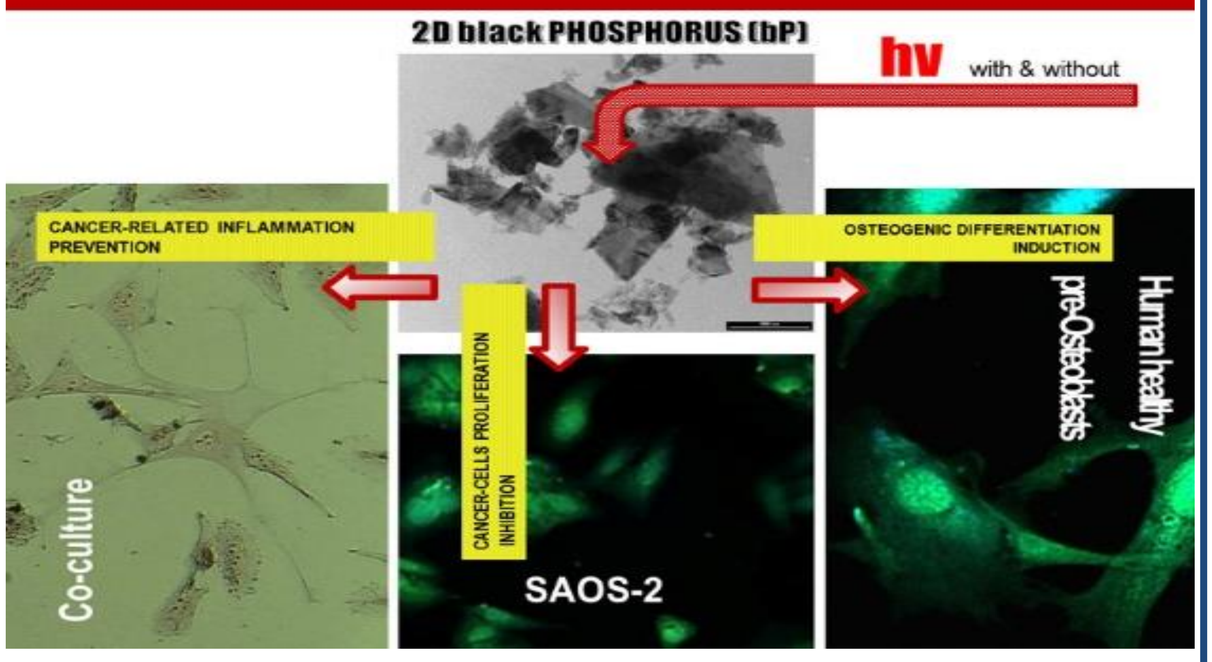


**Fig. 1. Selective anticancer potential of 2D BPNs against SAOS-2[3].**

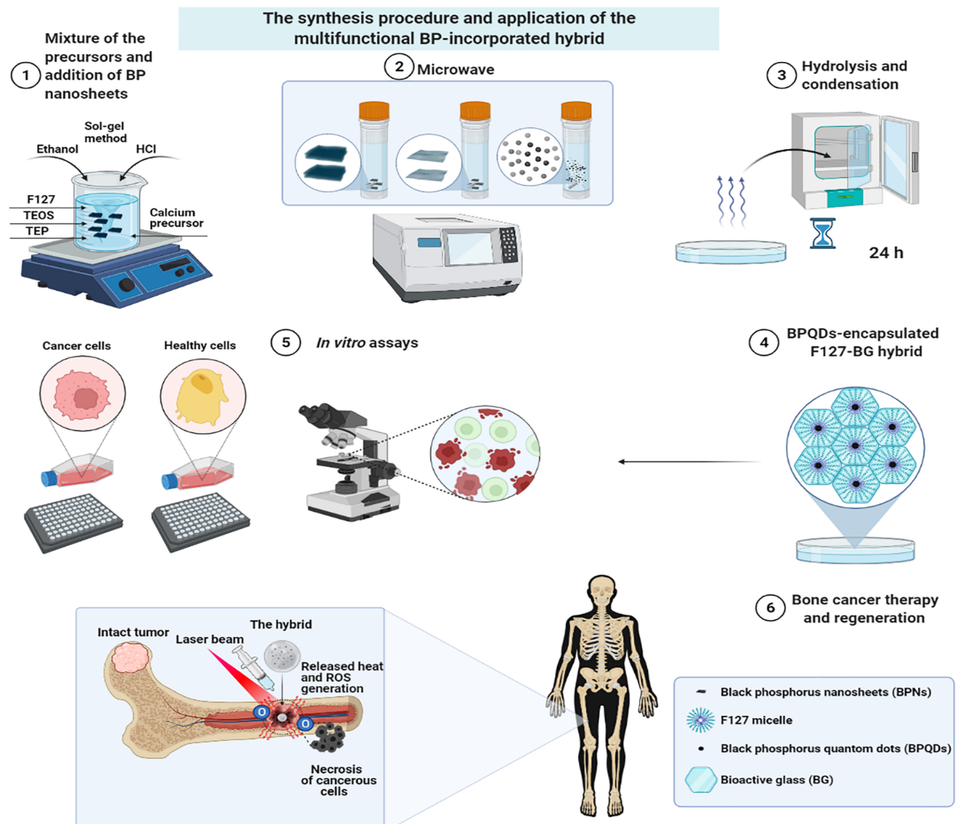


**Fig. 2. A schematic on the synthesis procedure and potential application of the organic/inorganic hybrid.**

## MATERIALS & METHODS

### *Synthesis and characterization of the organic/inorganic hybrid*

The hybrid, which is composed of F127/BG/BPQDs, was synthesized through a two-step synthesis strategy including sol-gel method and microwave irradiation (Fig. 2). It is noteworthy that the effect of F-127, BG composition, and microwave irradiation on the hybrid's cell compatibility was assessed *in vitro*. The TEM and SEM equipped with EDS and elemental mapping were adopted to characterize the physical and chemical properties of prepared hybrid. The cell compatibility and cytotoxicity against fibroblast and osteosarcoma cells were evaluated *in vitro*.

## Results and discussion

The SEM micrographs plus elemental mapping of the samples are shown through Fig. 3. It is visible that F127 resulted in the homogeneous distribution of all ions all over the BG, whereas the microwave had no effect on the distribution. The fibroblast cells showed better cell compatibility than the control when calcium concentration decreased, while osteosarcoma cells showed better performance when the ions concentration was intact. The cell viability of samples was assessed against fibroblast cells (Fig. 4). Compared to the control, FM had better cell viability

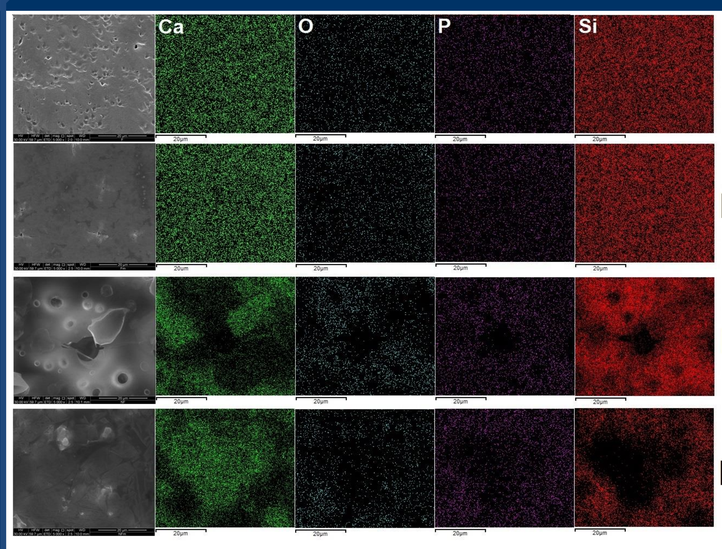


**Fig. 3. The SEM micrographs of BG (F), BG after microwave (FM), non-F127 BG (NF), and non-F127 BG after microwave (NFM).**

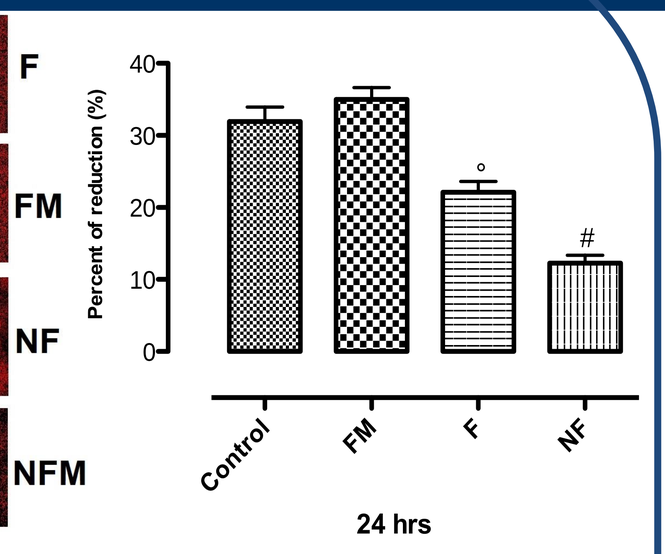


**Fig. 4. The cell viability of samples against L929 fibroblast cells after 24 h.**

than other samples followed by F and NF has shown cytotoxicity. The probable reason can be related to the microwave effect on the chemical stability of BG. Regarding the cell viability results here, the FM sample was chosen for further studies. The effect of different ions concentration on the cell viability of samples was also assessed. The FM sample with altered compositions was synthesized. The samples were FM (A), FM with half calcium (B), FM with half calcium and silica (C), FM with half calcium, silica, and phosphate (D), and half calcium, silica, phosphate, and F-127 (E). The cell viability was performed with the samples in three contents (20, 15, 5 mg) against osteosarcoma and fibroblast cells (Fig. 5).

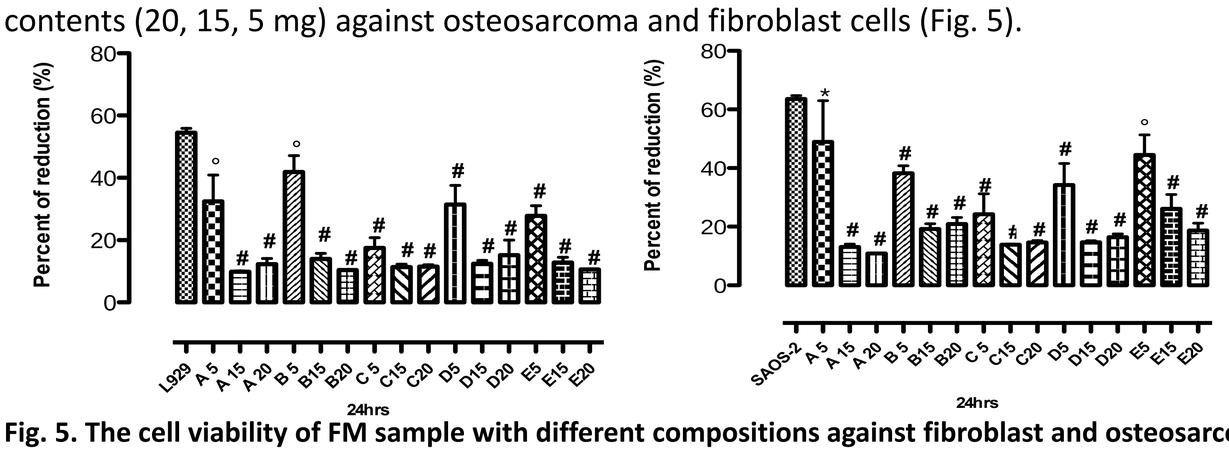


**Fig. 5. The cell viability of FM sample with different compositions against fibroblast and osteosarcoma cells for 24 h. *p≤0.05; °p≤0.001 and #p≤0.0001 vs Control.**

Nevertheless, the decrease in the hybrid weight from 20 to 5 mg caused an increase in the cell viability. The effect of microwave treatment on the F127/BG/BPNs physicochemical properties was assessed and shown through Fig. 6 (TEM, EDS, and Raman), where the BPNs were turned into BPQDs with a size less than 10 nm as the result of microwave irradiation. BPQDs with high absorbance in the NIR spectrum are of particular interest for diagnostic and therapeutic applications.

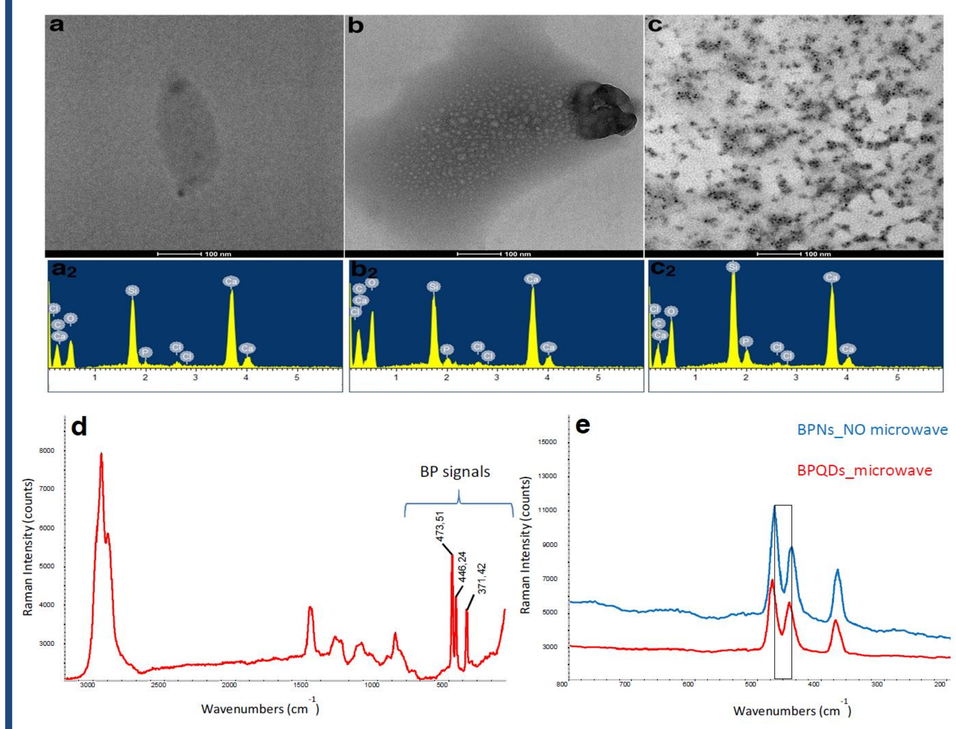


**Fig. 6. The TEM micrographs of F127/BG (a), F127/BG/BPNs (b), and F127/BG/BPQDs (c). The EDS analysis of F127/BG (a2), F127/BG/BPNs (b2), and F127/BG/BPQDs (c2). The Raman spectra of F127/BG/BPQDs (d) and BPNs and BPQDs (e).**

## CONCLUSIONS

An organic-inorganic hybrid composed of F-127/BG/BPQDs was developed. The effects of both F-127 and microwave irradiation on the cell compatibility of samples were assessed *in vitro*. It has been revealed that F-127 and microwave together made the hybrid's structure more stable resulting in the better cell compatibility than the untreated samples. The microwave treatment yielded BPQDs. The early studies paved the way for the future biological studies of F127/BG/BPQDs (in progress).

## REFERENCES

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## ACKNOWLEDGMENTS

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