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Is it Trendy to do Research on Graphene Based Materials for use in Regenerative Medicine?

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Abstract

Regenerative medicine requires adequate physical supports to facilitate appropriate cellular architecture, cell polarization and guidance and the improvement of the correct differentiation processes of embryonic or adult stem cells and induced pluripotent cells. Interdisciplinary collaboration between cell biologists, physicists and engineers is required to develop and successfully achieve the objectives of this pioneering research in tissue engineering. We try, in these lines, to highlight some of the technical and methodological issues useful in the research of graphene-based nanomaterial cytocompatibility, focused on its use in biomedicine. The general goal must be increasing standardization, precision and reproducibility of cellular assays.

We observed a direct relationship between the thickness and roughness of the graphene-based nanomaterials films and cell maturation. Therefore, the importance to find the most suitable thickness for inducing cell orientation, cell adherence and cell maturation. Because these types of nanomaterials are synthesized under highly controlled parameters, they might prove to be a powerful and reproducible platform for use in biomedical applications, such as tissue engineering and development of biocompatible devices.

Keywords: Graphene; Graphene-based nanomaterials; Regenerative medicine; Nanocrystalline; Formazan

Abbreviations: GBN: Graphene-Based Nanomaterials; MTT: Methylthiazolyldiphenyl-Tetrazolium

Introduction

Regenerative medicine requires adequate physical supports to facilitate appropriate cellular architecture, cell polarization and guidance and the improvement of the correct differentiation processes of embryonic or adult stem cells and induced pluripotent cells. Interdisciplinary collaboration between cell biologists, physicists and engineers is required to develop and successfully achieve the objectives of this pioneering research in tissue engineering. The materials most frequently used for those purposes are carbon derivatives and although there is abundant research with other materials, we consider that the most widely currently used type of materials are those comprised under the term graphene-based nanomaterials (GBN).

The interest in GBN research for use in biomedicine has grown within the last decade mainly due to the great potential of nanomaterials as components of biomedical devices. Their exceptional mechanical, electronic, and thermal properties together with the possibility to be tuned chemically to be biomimetic, attracted the attention of researches to incorporate these materials into new biomedical devices. Because of their physical properties as wettability, nanotopography, mechanical properties–stiff yet strong- and electrical conductivity [1], these materials offer better prospects for biological compatibility than other materials.

Nevertheless, graphene is a single monatomic layer of carbon atoms arranged neatly in the form of hexagons. Obviously, working with a perfect crystalline material with monoatomic thickness is still a challenge, so depending on the final application, there are very interesting materials derived from graphene. In the case of materials in which it is important to obtain certain surface properties, such as in osteogenesis and in neuronal growth, the properties of these surfaces will not only depend on the outer layer of material in contact with nerve cells, but also on the bulk material, in particular those of regions close

to the surfaces. That is why it is so important to differentiate among different types of GBN [2].

A larger number of projects have been carried out with GBN in bone regeneration [3,4] and to a lesser extent in skin regeneration [5,6], ocular engineering, nerve cell differentiation and cardiac tissue or musculoskeletal engineering [6]. But, before the exploitation of GBN, the biocompatibility of different types of graphene used for these purposes must be accurately assessed. This has always been our first concern when testing new materials for their possible use in biomedicine, such as this particular nanometer-thin nanocrystalline glass-like carbon film, composed of curved graphene micro flakes joined by an amorphous carbon matrix [7].

More than one methodological study has also been performed to improve the tests to evaluate any citotoxic response to GBN [8]. We clearly recommend analysing a basic battery of tests prior to standardize the experiments [7]. The metabolic activity of cells in presence of different types of GBN is commonly assessed for toxicity and proliferation measurements using methylthiazolyldiphenyl-tetrazolium (MTT) [9] and apoptosis/necrosis flow cytometry assay respectively. Indeed, MTT is a reagent that primarily determines the metabolic capacity of cells, as it measures the mitochondrial respiration process through the production of formazan (MTT is transformed into formazan by the mitochondria using the respiratory chain). The greater the presence of formazan in the supernatant, the grater the optical density of the supernatant will change and be detected and quantified in a spectrophotometer. For this reason, this technique has been widely used to quantify cell proliferation and viable activity of cells. In other words, the greater number of cells in the plate, the greater production of formazan, and therefore it indicates higher viability (dying cells are not viable and therefore do not transform MTT into the same amount of formazan). However, in the use of this assay we have a serious discrepancy like other authors [7,10] because not all cells have similar metabolic activity [9,11].

Oncogenic cells which have an unlimited capacity for division and are eternalized are by definition cells with higher proliferation and metabolic rates. But, on the contrary, osteoblasts and neurons do not behave in such a way; metabolism will depend on the functional capacity of the neural cells [12] and on the osteoblastic functional capacity in osteogenesis [13]. Both processes depend on cellular communication [6]. Nevertheless, cell proliferation and differentiation processes should not have a proportional effect. That is, the greater the differentiation of a neuronal cell the grater cell proliferation capacity decreases. Differentiation processes affect the proliferative capacity of cells.

It has been described very extensively in the literature that processes of quiescence [14] are in a direct relation with the differentiating mechanisms [15]. Moreover, in the case of nerve cells the proliferative capacity of an adult neuron is practically inexistent, so when the differentiation process ends, the adult neuron continues with the aging process (activation of senescence process) until dying. What we do know is that it could be replaced by another cell whose ability to differentiate had not ended up, like happens in astrocytes or astroglia.

For this reason, the fact that cells growing in GBN flakes or on top of GBN surfaces show a decrease in the amount of cells in the carbon containing systems, compared to the control, is an important result that supports the fact that our material favors the processes of differentiation [7,16]. When embryonic/transgenic origin cells are used we must consider that we are working with immortalized and undifferentiated cells and therefore, their division capacity is undetermined. For this reason they do not have a terminal growth pattern. They are not mature cells nor can they be considered adult cells.

In other words, they can be dividing eternally without control and also, the effect of GBN transforms the cells to differentiated-matured cells and this, in fact, represents an important phenomenon [7]. If cell morphology is checked, they show a purely fibroblastic-like structure in most cases. Instead, our GBN not only allows growth and adhesion of cells, but in turn differentiation and networking [7]. This capability is a very desirable fact for communication and survival of adult cells. In fact, GBN induce neuronal and osteoblastic differentiation or maturation without using any cell growth factors such as GDNF, BDNF neither Laminin matrix [17], that is, spontaneous growing is allowed.

For this reason, we think it is not suitable to compare a type of immature cell with another mature one in relation to the net percentage of cells. So this result itself is important for neuronal and bone regeneration; hence the relevance of our work. In relation to bone regeneration, it must be taken into account that the bone is a living organ, in constant interaction with the whole organism and with the rest of the organs. It is responsible for such important functions as regeneration, osmotic and hormonal control processes.

Therefore, the current orthopedic prosthesis, far from resembling and interacting with the organism, is inert reinforcements that do not facilitate cellular communication and interaction. Graphene, by its characteristics of permeabilization and absorption of minerals, proteins [18,19], etc, may be a suitable candidate for repairing osseous lesions or as a prosthetic material. In other words, graphene could behave as a "living prosthesis" proceeding from an inert material. This intrinsic characteristic of graphene makes it unique.

Considering the capacity of GBN to promote networking, rearranging the cells in such a way that their architecture changes but nuclear counting is smaller, make us think that it does not really have to be like that, since the cellular disposition seems to change from a monolayer to cell grouping. We have tried in this paper to highlight the technical and methodological

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issues useful in the research of GBN biocompatibility with a focus on its use in biomedicine. The goal is to achieve a methodology that increases standardization, precision, and reproducibility of cellular assays. Nevertheless, it is an objective to be completed through testing these materials in experimental animals, as concerns associated with the use of certain types of GBN in vivo still persist.

Conclusion

In light of our results in relation to a particular nanometer-thin nanocrystalline glass like carbon found to be biocompatible for SN4742 cells we encourage the research in graphene-based nanomaterials for use in regenerative medicine. These materials may provide an excellent physical support to grow cells and stimulate differentiation and neuronal and bone functional capabilities. We observed a direct relationship between the thickness and roughness of the films and cell maturation and therefore, the importance to find the most suitable thickness for inducing orientation, number of adhered cells, and cell maturation. Because these types of nanomaterials are synthesized under highly controlled parameters, they could offer a powerful and reproducible platform for use in biomedical applications, such as tissue engineering and development of biocompatible devices.

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