A Mini Review: Interaction of Graphene Oxide with Wharton's Jelly Derived Mesenchymal Stem Cells

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ABSTRACT Mesenchymal stem cells (MSCs) hold a great promise for tissue regeneration due to the ease in isolation, expanded capability, high plasticity, wide multi-lineage potential with attractive immunosuppressive properties and transplanted applicability. MSCs therapy relies on a large quantity of stem cells from derivative sources. The study of human Wharton's Jelly MSCs (hWJMSCs) from umbilical cord matrix has brighten the field of MSCs with the advantage of its availability, ethically free, easily harvested, non-invasive and accessible resources. Biomaterials such as Graphene Oxide (GO) can further enhance the yield of hWJMSCs while sustaining the proliferation potential, multi-potency and phenotype of the cells *in vitro* with potential applications in tissue engineering and regenerative medicine. The purpose of this mini review is to specify the unique properties of hWJMSCs and GO functionalization and the characterizations of hWJMSCs-GO cell-material interactions including morphology, proliferation, differentiation, and phenotype.

KEYWORDS: Graphene Oxide, substrate, Wharton's Jelly-derived Mesenchymal Stem Cells, functionalization, stemness.
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INTRODUCTION

Mesenchymal stem cells (MSCs), also known as marrow stromal cells, encompass a rare population of multipotent progenitor cells that possess high self-renewal ability and multipotential differentiation into tissues of mesenchymal lineages including bone, adipose, cartilage, tendon, skeletal muscle (Pittenger et al., 1999; Reyes et al., 2001). MSCs hold a great promise for tissue regeneration due to the ease in isolation, expanded capability, their high plasticity, wide multi-lineage potential with attractive immunosuppressive properties and transplanted applicability (Anderson et al., 2016, Zhao et al, 2016). MSCs therapy relies on a large quantity of stem cells from derivative sources (Dominic et al., 2006 and Upadhyay et al., 2016). The common sources of MSCs include umbilical cord (from the blood and Wharton's Jelly (WJ)), bone marrow (Zhao et al., 2015), placenta, fat, amniotic fluid (Tsai et al., 2004), and urinary membrane (Bharadwaj et al., 2011). In contrast to bone marrow MSCs, hWJMSCs could be isolated easily, have greater expansion capability, faster growth *in vitro*, and may synthesize different cytokines (Troyer and Weiss, 2009).

Schneider et al. (2010) reported that there is a need of an instructive biomaterial-based scaffold to direct stem cell (including WJMSCs) differentiation, proliferation, paracrine activity as well as regulation of extracellular matrix (ECM) deposition. The ability to dynamically vary cell–material adhesiveness (Collier & Mrksich, 2006; Kloxin et al., 2009), stiffness (Guvendiren & Burdick, 2012; Young & Engler 2011) and ECM degradability (Khetan, 2013) has recently induced readily observable changes in cell behavior. Stem cells in contact with materials are able to sense their properties, integrate cues via signal propagation and ultimately translate parallel signaling information into cell fate decisions (Murphy et al. 2014; Hiew et al., 2018).

Graphene-family nanomaterials include few layer graphene, graphene nanosheet, graphene oxide and reduced graphene oxide (Figure 1), have been studied in the area of biomedicine (Jastrzębska et al., 2012). Graphene Oxide (GO) is a unique material especially for *in vivo* implanted applications such as orthopedic therapies (Chen et al., 2012; Li et al., 2016). It is a chemically modified graphene, containing oxygen functional groups, namely carboxyl, epoxide and hydroxyl groups with amphiphilic properties (**Figure 1**). Chemical exfoliated GO of single or multilayer atomic sheets are highly potential for biomedical application due to its physicochemical stability, high electrical conductivity, tunable amphiphilicity (Liu et al., 2013; Kim et al., 2013; Saravanan et al., 2017), mechanical strength (Yang et al., 2016) and good biocompatibility. The unique properties of GO also support the cellular behavior such as cell adhesion, spreading, proliferation, differentiation and polarization (Nishida et al., 2016). Lee et al. (2011) reported that Graphene and GO show differences in inducing stem cell differentiation. Akhavan et al. (2013) observed an excellent proliferation of MSCs on reduced graphene oxide nanogrids (rGONG) through rapid asteogenic differentiation that is attributed to both capability of the rGONG material in high adsorption of the chemical inducers and the physical stress induced by surface topographic features of the nanogrids. The key advances of graphene oxide on controlling stem cell growth and various types of stem cell differentiation and the possible molecular mechanisms of graphene oxide in controlling stem cell growth and differentiation have been reviewed by Halim et al. (2018).

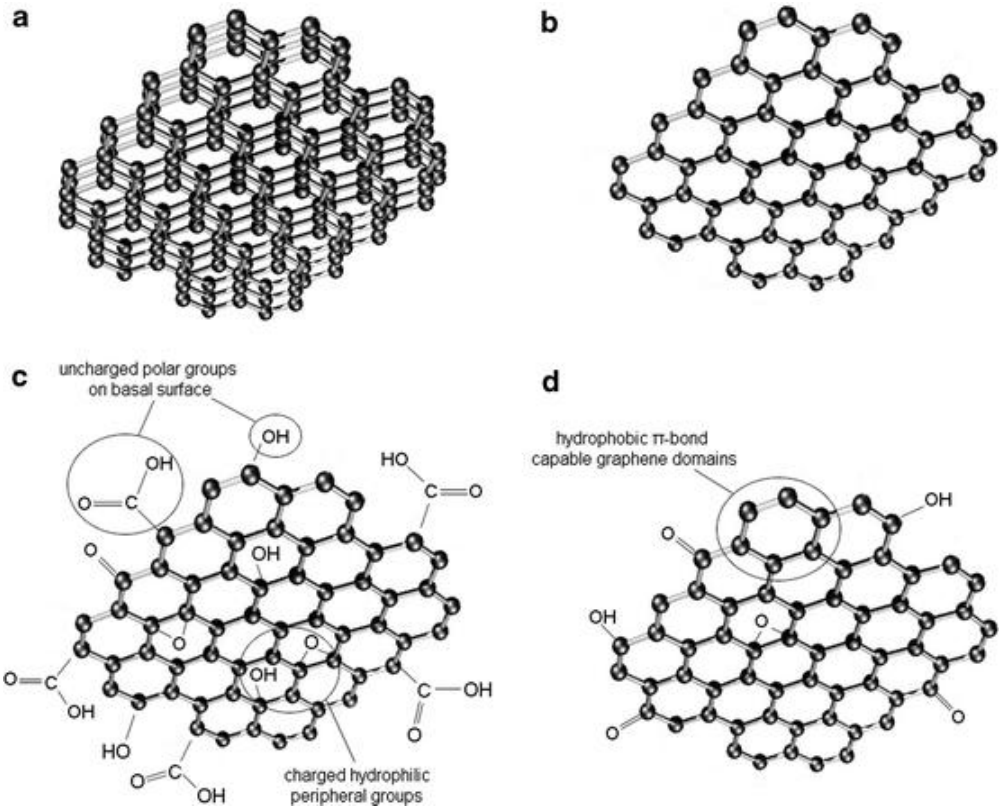

Figure 1. Structure of graphene-family nanomaterials. (A) Few- layered graphene, (B) Graphene nanosheet, (C) Graphene oxide, (D) Reduced graphene oxide. Source: Jastrzębska et al. (2012).

PREPARATION AND FABRICATION OF GRAPHENE OXIDE

The oxidation of graphite is the thresholds to produce GO. Potassium permanganate (KMnO4) with a ratio of 9:1 mixture of H_2SO_4/H_3PO_4 is used to improve the efficiency of the oxidation process (Zhu et al., 2012). The method possesses advantages like free generation of toxic gas and ease temperature control throughout the synthesis process. Mechanical exfoliation of water using sonication is applied to produce a single or few layers of GO (Lee et al., 2011). This process is

frequently used by scientist because it is cheaper, simpler, more efficient, better in both quantity and quality compared to the current method used in industries.

GO thin film can be prepared by depositing small amount of GO solution onto a substrate. There are many methods to prepare GO thin film such as spin -coating (Yamaguchi et al ., 2010), drop-casting (Gómez-Navarro et al., 2007), dip-coating (Wang et al., 2008), spray coating (Gilje et al., 2007) and Langmuir-Blodgett (L- B)/Langmuir-Schaefer (Gengler et al., 2010). The film uniformity, surface morphology, thickness and surface coverage of GO thin film are mainly depending on the deposition methods and parameters used. The surfaces of glass substrates can be treated with acid or (3-aminopropyl)triethoxysilane (APTES) prior to deposition for improvement of adhesion on glass substrates.

PROPERTIES OF GRAPHENE OXIDE

GO is also known as a flake material that can be visible in yellowish suspension. Physically, GO suspension is soluble in water and will turn from dark brown to light yellowish regardless of any concentration. The solubility and dispersibility of GO in water and organic solvent is due to the ionizable edge –COOH affected by pH change. GO synthesized by oxidation and exfoliation of graphite (Liu et al., 2013; Rosa et al., 2016; Upadhyay et al., 2016) is with excellent amphiphilic properties.

The unique properties of GO especially as a carbon-based material leads to the elevation of hydrophilicity and large surface areas to be loaded with large energy. GO is widely utilized as electrical insulator, physicochemical stability, mechanical strength, and it is biological compatible. Particularly, GO is made up of three Reactive Oxygen Functional Group (ROFG), which has the properties of electrical insulator including that of the epoxide, carboxyl and hydroxyl groups as shown in Figure 1C. Electrically, GO is a great insulating material due to the characteristics of saturated sp3 orbital coordinate, the missing carbon atom and negatively charged density species bound to carbon engendered GO as a super capacitor material (Li et al., 2015; Sharma et al., 2015; Becerril et al., 2008).

GO can be dispersed in physiological media making GO purposely applied in biomedical fields (Cote et al., 2011; Kim et al., 2010) and potentially used for drug delivery (Saravanan et al., 2017). Other than that, GO (Wang et al., 2011) is a biocompatible material that organizes the cellular behavior concisely as reported by Luo et al. (2011) in which the GO-incorporated Poly(lactic-co-glycolic acid) known as PLGA enhances the cellular metabolism and osteogenic differentiation through the unique surface interaction. Due to these unique properties, GO is preordained to be a favour candidate in its functional modification. GO in low dose (0.1mg/ml) and mild dose (0.25 mg/ml) are enough to activate cell growth which is considered as high biocompatibility (Luo et al., 2015; Wang et al., 2011).

WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS

Fetal stem cell is a possible derivative from multiple tissues and organs like placenta (In't Anker et al., 2004), amniotic fluid (Tsai et al., 2004), umbilical cord (Martin-Rendon et al., 2008), and umbilical cord blood (Jang et al., 2006). Bharti et al. (2018) concluded that hWJMSCs derived from all the parts of umbilical cord are valuable sources and can be efficiently used in various fields of regenerative medicine. MSCs parentage is from a layer of mesoderm that has been proven to have high capacity for self-renewal and great in multi-lineage differentiation into adipocytes, osteoblasts,

chondrocytes and smooth muscle cell (Zhao et al.; 2015; Pittenger et al., 1999; Wang et al., 2004; Kundrotas, 2012; Kalbacova et al., 2010; Zeddou et al., 2010; McMurray et al., 2011; Ku & Park, 2013; Kalaszczynska & Katarzyna, 2015; Koroleva et al., 2015).

True MSC must at least have three minimal criteria. Firstly, MSC must be plastic-adherent when maintained in standard culture conditions. Secondly it must be enable to express CD105, CD73 and CD90, and lacking expression of CD45, CD34, CD14 or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules. Lastly MSC must be able to split osteoblasts, adipocytes and chondrocytes *in vitro* (Dominici et al., 2006).

The study of human Wharton's Jelly from umbilical cord matrix has brighten the field of MSCs with the advantage of its availability, ethically free, easily harvested, non-invasive and accessible resources (Edwards, 2007; Nagamura-Inoue & He, 2014). Successful stem cell therapy requires billions of hWJMSC in their application. However, there is an inevitable factor which may lead to the loss of multi-potent ability and this depends very much on the cell senescence. Thus, effort is needed towards the development of an innovative method such as the use of biomaterials that can enhance the yield of hWJMSC in sustaining the proliferation potential and multi-potency of the cells.

CHARACTERIZATION OF hWJMSCs-GO INTERACTIONS

Despite the various studies on MSCs and GO interactions, there are relatively fewer reports on the hWJMSCs-GO interfaces. Herein, this mini review summarizes the characterizations of hWJMSCs-GO cell-material interactions including morphology, proliferation and differentiation, and phenotype.

GO has demonstrated fascinating interactions with living cells. The distinct ability of GO with great loading capacity and higher electrostatic interaction presence in the oxygenated groups functionalization plays a major role in facilitating stem cell adhesion and attains higher adsorption of proteins serum, induced cell proliferation and osteoblast differentiation (Lee et al., 2011).

The hWJMSCs retain their morphology of fibroblast-like or in the start-like shape on GO as analyzed using microscopic methods (Nayak et al., 2011; Puah et al., 2018, **Figure 2**). The short-term *in vitro* culture studied by Puah et al. (2018) showed an evidence that hWJMSCs were able to grow on GO-coated substrate at day 1 and 6 with no significant difference on the proliferation of GO compared to the glass cover slip control as analyzed using PrestoBlue™ assay and no apoptosis was observed (Puah et al., 2018). Recently, Puah et al. (2019) reported that hWJMSCs growth on multilayer GO films (6.25 μg, 12.5 μg and 25 μg) prepared using a simple and inexpensive drop-casted fabrication strategy enhanced the osteogenic differentiation. Parallel to the study, hWJMSCs successfully prolonged its growth on the GO substrates up to passage five (Hanim et al., 2018). They concluded that GO shows excellent performance in regulating the self-renewal and differentiation processes of stem cells including MSCs in which GO has the ability to control the differentiation of MSCs towards osteoblasts and adipocytes.

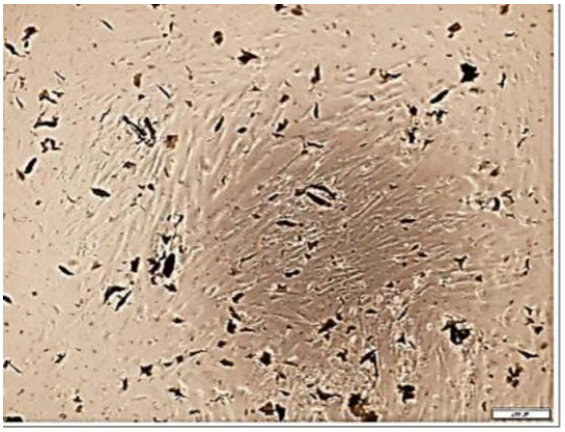

Figure 2. Fibroblast-like morphology of hWJMSC on GO-coated coverslip. Source: Puah et al. (2018).

The size of the GO flakes and the reduction level of GO have been considered as important factors determining the most favourable surface for hWJMSCs growth as reported by Jagiełło et al. (2019). Their analysis demonstrated that hWJMSCs cultured on all the tested GO and rGO scaffolds showed no alterations of their typical mesenchymal phenotype, regardless of the reduction level and size of the GO flakes. They concluded that GO scaffolds and rGO scaffolds with a low reduction level exhibit potential applicability as novel, safe, and biocompatible materials for utilization in regenerative medicine.

CONCLUSION

GO-based biomaterial is potentially relevant in hWJMSCs proliferation and differentiation with no alteration on the mesenchymal phenotype. This promises the GO applications either by direct implant injection or indirectly in biomedical and tissue engineering fields for regenerative medicine and cell therapy.

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