Application of Graphene, Graphene oxide and their derivatives as Wound healing: A Brief Review

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ABSTRACT
Background: Wound is this basic problem for everyone's life. Tissue engineering using graphene is the current issue to heal the wound faster and effective way. **Objective:** This review article documents the use of all forms of graphene as wound healing agent, method of preparation of graphene with other biocomposites and comparison between all form of graphene and their composites to find best suitable biomaterial as wound healing agent in present and future. **Results:** Graphene is used as wound healing in different forms such as graphene oxide (GO), reduced graphene oxide (rGO), graphene with polysaccharides nanocomposites, graphene foam (GF), graphene hydrogels, introduction of metal with graphene hydrogels, using biological scaffold such as fibrin and collagen with graphene, graphene nanotubes, nanofibers, graphene oxide collagen scaffold with incorporation of wound healing agent such as curcumin and graphene oxide (GO) nanoflakes. **Conclusion:** Graphene is futuristic biomaterial in wound healing. A further study is required to investigate the toxicological studies on graphene based biomaterial.

KEYWORDS: Graphene, Wound healing, Tissue Engineering, Nano-material; Polymer

INTRODUCTION

Skin is a fundamental common boundary organ for shielding inner organs from the outside condition and averting body parchedness. Any damage to skin causes microorganisms would enter easily and start to form colonies thereby leading to severe wound infection. [1][2]. Skin wound healing is a complex process involving many cell types and processes, such as epidermal, fibroblastic, and endothelial cell proliferation, cell migration, extracellular matrix (ECM) synthesis, and wound contraction regulated by an array of cytokines and growth factors [3] [4][5][6]. In spite of all the exploration and big developments in the commercially available skin substitutes, the regeneration of functional skin remains a challenges [7][8]. At present accessible method for wound healing fall over different problems, such as wound contraction, scar formation, and poor integration with host tissue [9]. Tissue engineering has developed as a promising way to deal the loss or defective wound area, thereby improving wound healing process such as using different method includes scaffolds, cells and nan-material alone or in combination [10]. In recent years, graphene which is composed of two-dimensional single or few layers of sp^2-bonded carbon sheet has attracted great interest as wound healing materials [11]. This review article documents the use of graphene and graphene nanocomposites as wound healing in different forms.

Graphene composites:

    Graphene foam (GF) scaffold (3D) laden with mesenchymal stem cells (MSCs) from the bone marrow to enhance skin wound healing [12]. Moisture and humid environment is a huge problem in handling wound. Thus to find a ideal hydrogels, Ag/graphene composites with acrylic acid and N, N ′-methylene bisacrylamide cross-linked were prepared [13]. Chitosan Polyvinyl Alcohal nanofibers containing graphene were found to be effective in mouse and rabbit. It also serve as effective antibacterial nanofibers [14].

Graphene oxide composites:

    Poly(vinyl alcohol)/chitosan/graphene oxide biocomposite nanofibers (PVA/CS/GO) could be a promising tissue engineering wound healing material [11]. Chitosan (CS) had used as biomaterial alone in bone regeneration but met with limited success therefore Chitosan (CS), gelatin (Gn) and graphene oxide (GO) scaffolds were designed as improved version in bone regeneration [15]. Hybrid hydrogel membranes composed of reduced graphene oxide (rGO) nanosheets and a poly (vinyl alcohol) (PVA) matrix would be a promising future in biological applications, such as transdermal therapy and wound healing [16]. Preparation of collagen-fibrin biofilm with the help of graphene oxide proved to be promising in healing wound on animal model [17]. Collagen functionalized nano graphene oxide (CFNGO) with induction of drug such as curcumin was evaluated as effective in open wound model [18]. Incorporation of silver with reduced graphene oxide may increase the wound healing effect and prevent infection at same time [19]. A similar work was carried out using silver and reduced graphene oxide in the presence of silver chloride and was found to be effective in burn wound model [20]. Preparation of near infrared (NIR) laser mediated surface activation of graphene oxide nanoflake was found as effective wound healing agent [21].
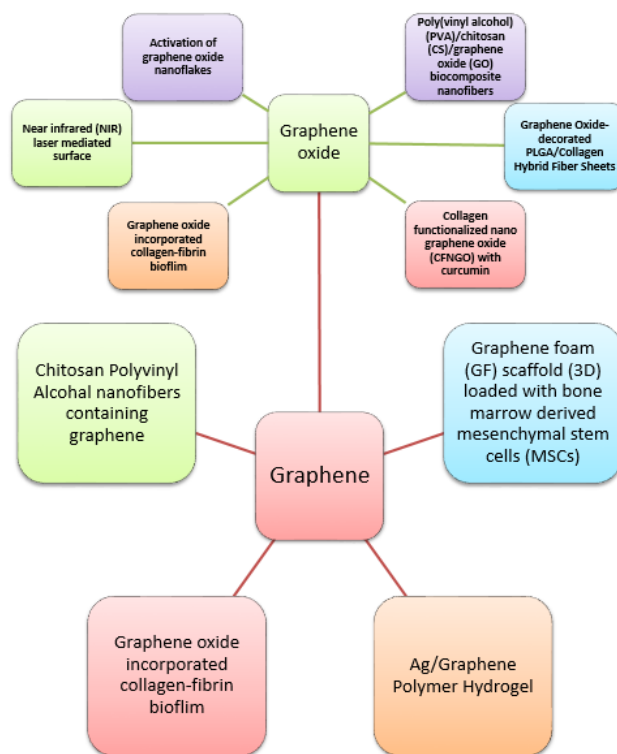
Fig. 1: Graphene and their derivatives in wound healing

Table 1: Method of preparation of sample in wound healing

Graphene and its derivative	Synthesis of graphene and graphene oxide	Preparation of suspension/sample	Reference
Poly(vinyl alcohol) (PVA)/chitosan (CS)/graphene oxide (GO) biocomposite nanofibers	Hummers method	Chitosan was prepared after EBI treatment. PVA solution was prepared by dissolving in DI water at 90.8C for 24 h. The homogeneous aqueous solution was obtained by mixing 2 g PVA and 5 g CS solution under vigorously magnetic stirrer. Then glyoxal solution (6 wt% with PVA) was added as cross-linking agent while the pH of the system was adjusted at 2–3 by phosphoric acid. Furthermore, dried graphene oxide was dispersed in DI water to suspensions (20 mg/ml) with the help of mild sonication for 1 h, then was added to the PVA/CS system and continually stirring for 2 h. electrospun at 18 kV by maintaining a tip-to-collector distance of 16 cm. As-spun PVA/CS/GO nanofibers were collected in Teflon paper; put into the oven under alcohol	[11]

		vapor at 50.8C for 24 h, and then cured at 120.8C for 10 min.	
Graphene foam (GF) scaffold (3D) loaded with bone marrow derived mesenchymal stem cells (MSCs)	3D graphene foams were prepared by chemical vapor deposition method with Ni foams template	The samples were firstly put into a FeCl₃ (1M) solution for at least 72 h at room temperature. Then the obtained 3D-GFs were rinsed sequentially with 1, 0.1, and 0.01 M HCl solutions, followed by rinsing with running water for at least 72 h to remove the etching agents. After sterilization by 75% alcohol, the 3D-GFs were successively soaked into sterilized PBS buffer and coated with laminin (5 mg/ml, Sigma) solution in PBS for at least 4 h at 37 °C. Just before cell seeding, 3D-GFs were soaked in the medium overnight. MSCs cultured on the 3D-GFs exhibited excellent cell adhesion and formed a 3D network	[12]
Graphene Oxide-decorated PLGA/Collagen Hybrid Fiber Sheets	Hummers and Offeman method.	20% (w/v, 200 mg/mL) poly(lactic-co-glycolic acid (PLGA) and 0.1% (w/w, 0.2 mg/mL) GO of the total PLGA weight were dissolved in 1,1,1,3,3,3,-hexafluoro-2-propanol solvent to form the first solution. The GO solution in HFIP was prepared by sonicating GO for at least 2 h to uniformly disperse GO particles. 8.5% (w/v) Collagen (Col) was dissolved in HFIP to form the second solution. As the first solution, the GO-PLGA solution was placed in 5 mL syringe fitted with a 25 G needle. A syringe pump was used to feed the GO-PLGA solution into the needle at the flow rate of 0.5 mL/h. The 10 kV positive voltage by a highvoltage power supply and a 11 cm working distance between a needle tip and a collecting drumwere adopted for the electrospinning process. The second solution containing Col was delivered to the 5 mL syringe fitted with a 21 G needle and pushed to the needle tip at the flow rate of 0.5 mL/h with another syringe pump. A voltage of 10 kV was applied and the working distance was 12 cm. The GO-PLGA/Col hybrid fibers were collected on a rotating drum wrapped with an aluminum foil. The rotating speed of the grounded drum was 20 rpm. Collected GO-PLGA/Col hybrid fiber sheets were subsequently vacuum-dried to remove any residual solvents.	[22]
Ag/Graphene Polymer Hydrogel	Hummers method	Acrylic acid (AA) and N , N ′-methylene bisacrylamide (BIS) were added to GO or Ag–graphene dispersion and stirred for 30 min in an ice-water bath to afford a dispersion. The dispersion was poured into a petri-dish with a diameter of 10 cm, and then the petri-dish was put into an oven to allow further polymerization at 65 °C for 4 h. Upon completion of polymerization, the hydrogels were peeled from petri-dish and washed with water to remove impurities.	[13]
Graphene oxide incorporated collagen-fibrin bioflim	Hummers method	Graphene oxide (GO) was further dialyzed for 24 h using water and then it was dried at 60°C. The powdered GO was further sonicated to get a well dispersed solution with different ratios. Collagen was isolated from chrome containing leather waste. The collagen was further purified by dialyzing against 0.1M acetic acid and distilled water respectively for 24h. The sample was freeze-dried and used as such. Physiologically clotted crude fibrin was separated from fresh blood by churning. Fibrin further purified by wet precipitation method. The sample was freeze-dried and used as such. Collagen-Fibrin (CF) films were prepared by the mixing both of them. CF composite which exhibited better tensile strength was selected and further mixed with GO to prepare CFGO films. Ethylene glycol was added as plasticizing agent. This mixture was poured into polythene trays (measurement 12 cm x 7.5 cm) and dried at room temperature (30˚C) to get CFGO in sheet form.	[17]
Chitosan Polyvinyl Alcohal nanofibers containing graphene	Micromechanical cleavage	Polyvinyl alcohol (PVA) was dissolved in distilled water (DW) and Chitosan (CS) was dissolved in DW. A PVA-DW solution was mixed with a CS solution in a volume ratio 70 : 30, and the solution was stirred for 30 min at room temperature. Second, graphene and N,N-dimethylacetamide (DMF, same weight as PVA) were added into the mixed solution, which was then subjected to ultrasound stirring treatment for about 30 min. Third, the mixed solution was then subjected to the electrospinning experiments. The electrospinning process was carried out at a voltage of 32 kV with a needle–collector distance of 8 cm..	[14]
Collagen functionalized nano graphene oxide (CFNGO) with curcumin	Hummers method	Graphene oxide (GO) solution was prepared with the addition of 100 mg of GO in 50 ml of 0.1M 2-(N-morpholino)ethanesulfonic acid (MES buffer) to maintain the pH of the solution at 6.5, and then it was treated with a probe sonicator set at 30% intensity for 30 min in an ice bath. To activate the carboxyl groups of the GO flakes, N-(3-dimethylaminopropyl-N0-ethylcarbodiimide)hydrochloride, and N-hydroxysuccinimide were added into the GO solution (50 ml) at a GO : EDC : NHS molar ratio of 1 : 2 : 2 and stirred with a magnetic bar for 24 h. About 0.5 g of fish scale type I collagen was dissolved in 50 ml of 1% acetic acid solution and added to the EDC–NHS activated GO solution, reaction was allowed to proceed further for another 24 h at room temperature to obtain the final product of collagen functionalized NGO (CFNGO). Curcumin loaded CFNGO was prepared by a simple, noncovalent interaction method. The loading of curcumin onto CFNGO scaffold was carried out by mixing 10 ml of curcumin solution (60 mg of curcumin) in acetone with 60 ml of a freshly prepared solution of CFNGO (60 mg of CFNGO scaffold) with constant stirring for 24 h, at room temperature. The suspension was then squeezed through a muslin cloth to remove any precipitate formed during the process and finally the solution was lyophilized.	[18]

Near infrared (NIR) laser mediated surface activation of graphene oxide nanoflakes	Hummers method	-	[21]

Conclusion:

Graphene is a carbon crystalline hexagonal lattice with amazing physical and chemical properties comprising of high tensile strength and extreme chemical stability [23] [24]. It is used in different form to improve the wound healing, enhancing the rate of wound contraction and reducing scar formation. Toxicological studies on biomaterial such as dermal toxicity, carcinogenic toxicity, allergenicity, genotoxicity is yet to be performed in most of discussed graphene based nanomaterial.

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