Materials Science and Engineering C 82 (2018) 323–329

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Cell attachment evaluation of the immobilized bioactive peptide on a nanographene oxide composite

Behzad Adibi-Motlagh [a], Abbas Sahebghadam Lotfi [a,*], Aram Rezaei [b], Ehsan Hashemi [c]

[a] Department of Clinical Biochemistry, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
[b] Nano Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[c] National Research Center for Transgenic Mouse, National Institute of Genetic Engineering and Biotechnology, P.O. Box 14965-161, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form 25 April 2017
Accepted 7 May 2017
Available online 17 May 2017

ABSTRACT

The immobilization of bioactive peptides as key molecules in numerous biological and physiological functions holds promise for designing advanced biomaterials. Graphene and its derivatives, having unique physicochemical properties, have brought considerable attention in the life sciences. In this regard, the chemical manipulation of the graphene surface with bioactive peptides opens a new horizon to design bioactive materials for a variety of future nanobiotechnologies. In this study, the first straightforward strategy for the covalent immobilization of the cell-adhesion peptide onto the graphene surface based on the Ugi four-component assembly process (Ugi 4-CAP) will be presented. The modified adhesion motif peptide, as an amine component in the presence of formaldehyde, cyclohexylisocyanide and carboxylated-graphene (G-COOH), was adopted in a four component reaction to fabricate a peptide-graphene (Peptide-G) biomaterial in water as a green solvent at an ambient temperature. The amino functional groups corresponded to the modified adhesion motif peptide and were immobilized onto the graphene sheets, which were quantified by the Kaiser test. The sheets were characterized by further analyses with FT-IR, AFM, UV–vis, Raman and thermogravimetric analyses. The Peptide-G biomaterial showed excellent biocompatibility. In addition, the Peptide-G treated surface, due to the presence of RGD on the surface of the graphene, significantly accelerated the proliferation of human mesenchymal stem cells (hMSCs) at a better rate regarding the tissue plate.

1. Introduction

Biomaterials are synthetic materials that have been engineered to interact through their interface with biological systems for therapeutic and diagnostic purposes [1]. Biocompatibility, mechanical properties, responsiveness and specificity of biomaterials are the crucial factors in designing materials for specific medical applications. To this end, scientists are creating new materials which improve the physicochemical interactions between the biomaterial and the physiological environments by an anchor specific functionality on the surface of the biomaterials [2,3]. Moreover, in recent years, the advents of nanotechnology and the integration with engineering materials and chemistry have revolutionized the medical sciences and have provided the capacity to design and develop nanobiomaterials for therapeutic and diagnostic applications [4]. The surface modification of the biomaterials by grafting peptides and proteins is a useful strategy to alter the physical and chemical properties of the original materials [5].

In the context of design and development of functionalized biomaterials, several methods have been investigated for the immobilization of the peptides and biomolecules onto the biomaterials. These involve multiple steps in nature with difficult workup procedures and are developed for some specific materials. Normally, most of the general methods can be classified into two major groups: physical adsorption and chemical binding. Using the metal ion affinity chromatography (IMAC) technique, Schmidt et al. reported the non-covalent immobilization of polyhistidine-tagged proteins [6,7]. Mac Beath et al. utilized the covalent non-site-specific immobilization strategy for printing proteins as microarrays for high-throughput function determination. In this strategy, biomolecules bind to the sensing substrate through the stable amide bond based on carbodiimide chemistry [8]. Other immobilization methods involve the reaction between the diazobenzylidene-functionalized glass and heteroatoms with acidic protons [9], the Michael reaction between a maleimide-functionalized surface and thiols [10], oxime site-specific ligation [11], α-oxo semicarbazone ligations [12], native chemical ligation (NCL) [13], "click" chemistry [14], the Diels–Alder reaction [15], and the Staudinger ligation [16] under harsh chemical environments during modification and produce undesirable immobilization products; therefore, it would be highly desirable to achieve

* Corresponding author.
E-mail address: lotfi_ab@modares.ac.ir (A.S. Lotfi).

http://dx.doi.org/10.1016/j.msec.2017.05.039
0928-4931/

programmable chemical transformations in which the biomolecules with a diverse functionality anchor onto the surface of nanomaterials to access a high level of complexity in biomaterial engineering.

To address the aforementioned problems, a fundamentally novel approach for preparing a multifunctionalized biomaterial based upon graphene is proffered. Bioactive peptides in the presence of formaldehyde and cyclohexylisocyanide are assembled on the graphene surface according to a cascade of convergent chemical reactions to achieve the peptide-graphene (Peptide-G) biomaterial.

More recently, we reported a fundamentally novel approach to prepare multifunctionalized graphene composites by the Ugi four-component assembly process (Ugi 4-CAP) in water at an ambient temperature, and the efficiency and competency of Ugi 4-CAP as a comprehensive method was examined by a successfully immobilized lipase enzyme onto the graphene surface [17]. Currently, a large number of synthetic materials including glass, polymers, porous materials, and more recently nanosized materials with a wide range of unique physical, chemical, and mechanical properties are available for use in life science. Recently, carbon-based materials such as carbon nanotubes (CNT), graphene, diamond, and fullerenes have gained significant interest for providing a large opportunity to serve as next-generation biotechnologies [18].

Graphene is an atomically thin material that has generated tremendous interest over the past few years. This exponentially growing interest has been due to its remarkable physical, chemical and mechanical characteristics. These features include inter alia, excellent electrical and thermal conductivity, high surface area, high carrier mobility, intrinsic biocompatibility, ease of functionalization, and production [19]. The graphene based material, apart from applications in many fields of science running the gamut from chemistry to electronics, offers great potential for applications in the biomedical science such as drug/gene delivery [20], biological sensing and imaging [21], antibacterial materials [22], enzyme immobilization [23], and biocompatible scaffolding for cell culture and tissue engineering [24]. Nonetheless, one of the most predominant challenges for the material to have a medical application is the inadequate interaction between the synthetic substrates and cells. Therefore, chemical manipulation of the material with the biomolecules is a promising plan to create biomaterials for applications in the life sciences [25].

Peptides, short chains of amino acid monomers, play important roles in many biological and physiological functions of living organisms, such as neurotransmitters, antimicrobial, antioxidant, antithrombotic, hormones, or antibodies, and are involved in the progression of several diseases. Therefore, the surface modification of the biomaterial by grafting peptides and proteins is a useful strategy to change the biological properties of the original materials for reducing the adsorption of the unspecific protein and enhancing the biocompatibility of the biomaterials [26].

A straightforward and robust approach for creating the multifunctional biomaterials is the use of multicomponent reactions (MCRs). MCR as a diversity-oriented synthesis can cover a broader range of chemical space due to the three or more starting materials with a variety of functionalities which are combined in one the synthetic transformation. In addition to the highly convergent nature, another major advantage of these reactions is their ability to combine starting materials in a sequence of elementary steps based on a plan to access a high level of complexity [27]. One of the most important MCRs to generate high level of diversity and complexity was reported by Ivar Ugi in 1959 [28]. In the Ugi four component reaction (Ugi 4CR), Oxo-substrates, amines, carboxylic acids, and isocyanides were combined in a one-pot reaction to afford the diverse α-acylamino amides. This Ugi four-component reaction (U-4CR) furnishes the Ugi adducts by combining the remarkable functional groups of starting materials that can be used in the modern combinatorial chemistry [29].

In this paper, a one-pot and versatile method was posited based on the multicomponent reaction for the covalent immobilization of bioactive peptides onto a graphene surface to create a Peptide-G surface. The biocompatibility of the composite was examined by the MTT assay.

2. Results and discussion

In this work, a fundamentally novel approach to immobilize a cell-adhesion peptide onto carboxylated-graphene (G-COOH) covalently by the Ugi 4-CAP reaction is postulated. To this end, for applying the proposed methodology, an aqueous suspension of graphene oxide (GO) was prepared by the Tour method [30]. The GO possess oxygenated aliphatic regions (hydroxyl, epoxy, carbonyl, and carboxyl functional groups) rendering it suitable for scaffold use in the biomaterial application and providing a handle for chemical manipulation to create new hybrid biomaterials [30]. Furthermore, the density of the carboxyl group on the basal plane of the graphene sheets was enriched by chloroacetic acid under basic conditions to produce a carboxylated-graphene composite (G-COOH) (Scheme 1) [17].

In the following section, it is shown that the modified peptides as an amine component in the presence of formaldehyde, cyclohexylisocyanide and G-COOH came together in water in a single reaction vessel to construct the Peptide-G composite. The elicited biomaterial hybrid based on graphene was able to disperse well in the aqueous solution (Scheme 2). We applied the quantitative Kaiser test in DMF to identify the amount of free amino groups on the surface of graphene that revealed the degree of functionalization [31]. For GO and G-COOH, a negative test value was obtained. However, after the functionalization of graphene by the bioactive peptide, the amount of amine function in Peptide-G1, Peptide-G2 and Peptide-G3 were calculated to be 78, 195 and 401 μmol/g of graphene, respectively.

The Peptide-G composite obtained were characterized by various techniques such as FT-IR, UV–Vis, Raman spectroscopy, AFM and TGA. FT-IR is the most direct method to characterize the functional groups in the graphene and its derivatives. The GO showed the characteristic peaks pertained to OH, C═O, C═C and C—OH functional groups at 3380, 1730, 1622, 1360 cm^{-1}, respectively [32]. In the G-COOH samples, after the oxidation of GO with chloroacetic acid in the presence of KOH, some new vibrational peaks appeared. The strong peaks located at 2852 and 2925 cm^{-1} could be assigned to the symmetric and asymmetric stretching modes of the —CH_2— groups. The peak at 1575 cm^{-1} was due to the skeletal vibration of C═C, and the deformation vibration of the —CH_2— groups appeared at 1370–1410 cm^{-1}. Moreover, the peak at 1217 cm^{-1} corresponding to the vibration mode of the epoxide groups disappeared after conversion to C—O—CH_2COOH groups [17]. To investigate the performance of the Ugi 4-CAP for multifunctionalization of graphene with the bioactive peptide, the Peptide-G composite was scrutinized by FT-IR spectroscopy. In Fig. 1, the presence of the stretching vibrations of the N—H bonds was confirmed by the broad peak at approximately 3231–3563 cm^{-1}. Additional peaks corresponding to the symmetric Vs (CH_3), asymmetric *Vas* (CH_2), asymmetric *Vas* (CH_3), and symmetric Vs (CH_2) appeared at 2952, 2920, 2866, and 2850 cm^{-1}, respectively. The amide carbonyl-stretching mode (amide I vibrational stretch) was assigned at 1625–1732 cm^{-1}; furthermore, the new peak at 1579 cm^{-1} could be attributed to the amide II vibrational stretching mode (coupling of N—H in-plane and C—N bond). While the C—N stretching vibration was revealed by broad peaks at 1227 and 1377 cm^{-1}, the bond at 1456 cm^{-1} was correlated with methylene scissoring [17,33–35]. Overall, these results confirmed the success of the multicomponent reaction to produce hybrid biomaterials.

UV–Vis spectroscopy was carried out to scrutinize the functionalization of G-COOH with the bioactive peptide through a multicomponent process. The G-COOH exhibited characteristic a peak at 258 nm, which might be germane to the π–π* transitions of the aromatic rings. However, the Peptide-G displayed a maximum absorption at