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Mass-production of fluorescent chitosan/graphene oxide hybrid microspheres for in vitro 3D expansion of human umbilical cord mesenchymal stem cells

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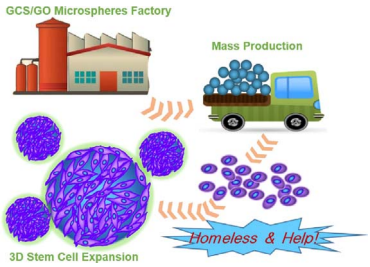
GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we designed a simple and low cost acid-dissolved/alkali-solidified self-sphering shaping method (AASS) to automatically fabricate three-dimension (3D) stem cell expansion microsphere scaffolds in large scale to satisfy the urgent need for stem cells in tissue engineering and clinical medicine research. We chose chitosan as the main part of microsphere scaffold, graphene oxide of 3 wt% as the strength agent, and genipin as the fluorescence generator to fabricate fluorescent chitosan (CS)/graphene oxide (GO) hybrid microspheres. The diameters of the hybrid microspheres are about 400 μm with the diameter error less than 10%, which is suitable for stem cells spreading. These hybrid microspheres with good biocompatibility can support the cells' spread, growth and proliferation. After cultured for 5 days, the total number of cells on microspheres has almost increased fourfold. Most importantly, the microspheres can maintain the cell type. After cultured for 7 days, almost all cells still express main markers of human umbilical cord mesenchymal stem cells (HUMSCs). The hybrid microspheres can support long-time stem cell expansion because of their good mechanical strength, controllable degradability, and low expansion rate when soaked in media. Furthermore, their autofluorescence also makes observing and tracking the stem cells behavior on surface of microsphere scaffolds more convenient. This research provides a powerful method for mass-producing chitosan/graphene oxide hybrid microspheres for 3D stem cells expansion. This method is easy to be put into industrial production, and may have tremendous value in medicinal and clinical applications.

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1. Introduction

With the rapid development of tissue engineering and clinical medicine, stem cell therapy has been a focus of attention, attracting plenty of researchers. There has been a large number of researches on tissue engineering [1–5]. The insufficient number of stem cell sources is an important limiting factor in tissue engineering and clinical medicine. Therefore, designing an efficient stem cell expansion method is an important solution to increase the number of stem cells extracted from bodies [6–10].

Traditional two-dimensional (2D) cell culture in culture plates cannot meet the need. On the one hand, the quantity of stem cells is quite limited in a 2D culture system, and on the other hand, the microenvironment of 2D cell culture is quite different with that in the body. This difference will influence gene expression and signal transduction of stem cells, and make 2D cultured stem cells lose their biological feature and functions [11–14]. Therefore, three-dimensional (3D) cell culture may provide an improved way of producing stem cells in large quantities.

3D cell culture can provide living conditions close to the microenvironment in the body, which is beneficial for cell growth and proliferation [15]. Stem cells cannot extend to their normal morphologies are not appropriate for direct transplantation into body with the current 3D cell culture technologies, which do not require scaffolds including cell micro-aggregates [16,17], magnetic levitation [18], and hanging drop plates [19,20]. In addition, the fussy operation and high cost also make conventional 3D cell culture methods hard to popularize. 3D cell culture technologies based on 3D scaffolds composed of biocompatible materials create no need for cell dissociation from the scaffolds, and aggregated but also uniformly distributed stem cells can be transplanted to tissue defects directly by targeted delivery of microstructures, which is beneficial for cell viability and multipotency maintenance. Therefore, 3D stem cell culture scaffolds offer the advantage of being able to directly inject these biodegradable culture systems, allowing cell repopulation or augmentation of cell population through growth factor or drug release agents aimed at regeneration. Therefore, 3D stem cell culture is necessary for supplying stem cells with both high production rates and high quality.

There has been a number of researches on 3D cell culture using biocompatible scaffolds. Vineetha et al. reported a self-assembled nanostructured hydrogel for 3D chondrocyte cell culture [21]. Theresa et al. studied the cellular mechanobiology in three-dimensional culture using collagen-agarose matrices [22]. Michiya et al. provided a brief overview of current developments on 3D tissue fabrication technologies and their biomedical applications [23]. However, most of the scaffolds in existence are only suitable for tissue repair in specific situations, and are thus not the best for stem cell expansion and quantity production.

Chitosan (CS) and its composite have raised significant interest of researchers and has been put into a broad range of applications, such as food packaging, cosmetic, and water treatment [24–27]. In addition, CS is especially applied in drug delivery [28,29] and tissue engineering [30–32] because it is a biodegradable, nontoxic, and biocompatible naturally derived polymer. There have been large amounts of previous researches proving the chitosan application feasibility in cell culture and in vivo transplantation [33,34]. Moreover, in order to improve the mechanical properties, chitosan is always combined with graphene oxide (GO) as a reinforcing agent. GO is also a biocompatible materials which is widely used in biological medicine and tissue engineering [35,36]. GO is rich in hydrophilic functional groups, such as –COOH and –OH, therefore, GO possesses strong interfacial interaction with polymers, especially hydrophilic ones. There has been a great number of researches using GO as a two-dimensional (2D) filler for the preparation of mechanically enhanced polymer composites [37,38]. However, to date, 3D cell culture scaffolds, including the chitosan and graphene oxide composite we used in this study, are mostly in shapes of bulks or nanonetworks composed of nanofibers [39–41]. These scaffolds with custom shapes can be implanted onto specific defects, but cannot be put into quantity production due to the variety of tissue defects. Therefore, we aim to construct an effective and flexible 3D microsphere cell culture system suitable for mass production using CS, as microspheres are most suitable for cell overspread and proliferation, and can act as cell collectors and carriers.

We designed an acid-dissolved/alkali-solidified self-sphering shaping method (AASS) for rapid and facile production of chitosan/graphene oxide hybrid microspheres as 3D cell expansion scaffolds. We chose CS as the main component, graphene oxide (GO) as the strengthening agent, and genipin as fluorescence generator to fabricate genipin-crosslinked chitosan/graphene oxide (GCS/GO) hybrid microspheres. These hybrid microspheres have good compatibility with cells and can support the spread, growth and expansion of human umbilical cord mesenchymal stem cells (HUMSCs) with almost no negative effects on cells type and migratory behaviors. These microspheres also have controllable degradability, and a low expansion rate which ensures the stability of the scaffold in long-time tissue regeneration. Furthermore, autofluorescence also makes observing and tracking stem cells behavior on surface of microsphere scaffolds convenient. In summary, this research has provided a method for stem cell expansion in vitro, based on quick and mass production of GCS/GO hybrid microspheres as 3D cell culture scaffolds. This 3D human stem cells model will be a powerful technique for applications in tissue engineering and stem cell therapy.

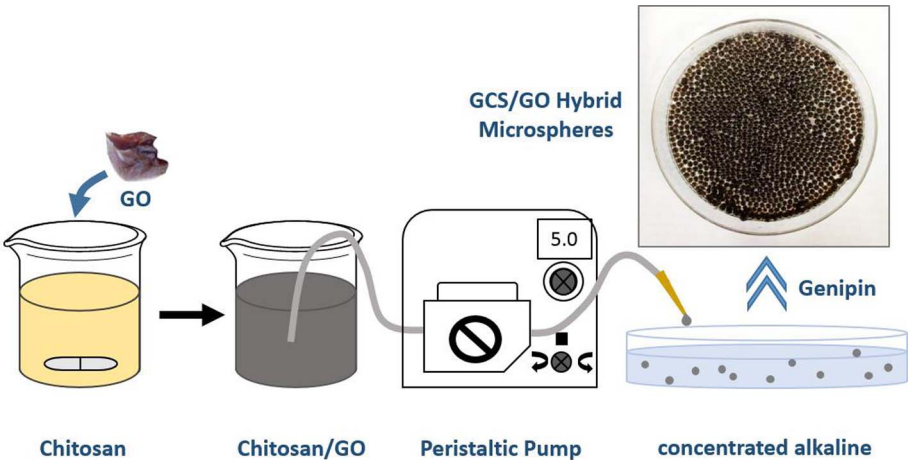

Fig. 1. Schematic of preparation procedure of GCS/GO Microspheres.