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Functionalizable hydrogel microparticles of tunable size and stiffness for soft-tissue filler applications

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ABSTRACT

Particle size, stiffness and surface functionality are important in determining the injection site, safety and efficacy of injectable soft-tissue fillers. Methods to produce soft injectable biomaterials with controlled particle characteristics are therefore desirable. Here we report a method based on suspension photopolymerization and semi-interpenetrating network (semi-IPN) to synthesize soft, functionalizable, spherical hydrogel microparticles (MP) of independently tunable size and stiffness. MP were prepared using acrylated forms of polyethylene glycol (PEG), gelatin and hyaluronic acid. Semi-IPN MP of PEG-diacrylate and PEG were used to study the effect of process parameters on particle characteristics. The process parameters were systematically varied to produce MP with size ranging from 115 to 515 μm and stiffness ranging from 190 to 1600 Pa. In vitro studies showed that the MP thus prepared were cytocompatible. The ratio and identity of the polymers used to make the semi-IPN MP were varied to control their stiffness and to introduce amine groups for potential functionalization. Slow-release polymeric particles loaded with Rhodamine or dexamethasone were incorporated in the MP as a proof-of-principle of drug incorporation and release from the MP. This work has implications in preparing injectable biomaterials of natural or synthetic polymers for applications as soft-tissue fillers.

1. Introduction

Injectable biomaterials are used in medicine to augment lost or compromised tissue in order to restore form and/or function to the affected region and represent a large market, worth billions of dollars annually. Examples of such biomaterials include dermal fillers, used to treat facial defects; viscosupplements, used to treat osteoarthritis of the knee; and biomaterials, used for augmenting the urethral wall to treat urinary incontinence. Novel injectable biomaterials continue to be developed to address this growing injectable-fillers market. Further, in a small subset of patients, adverse events, such as granulomas, swelling, erythema and nodules, occur even with US Food and Drug Administration (FDA) approved or cleared injectables [1–5]. There is thus significant interest in developing methods to prepare new and putatively better injectable biomaterials.

Most injectable fillers are particulate or break down into particulates upon injection [6]. The size, shape, mechanical properties and surface chemistry of the particulates are known to influence their in vivo performance. For example, silicone particles of less than 70 μm have been shown to migrate away from the periurethral injection site and cause complications [7]. The migration potential of an implant is important for determining its safety as per the FDA's guidelines for vocal fold applications [8]. Phagocytosis of polymethymethacrylate particles by macrophages is also size dependent [9]. Polymethylmethacrylate particles less than 50 μm in size or irregular in shape induce greater production of tumor necrosis factor alpha, an inflammatory cytokine, than particles that are spherical in shape or are between 50 and 350 μm in size in vivo [10]. Particles with acute angles, such as triangles, also induce a greater foreign body response than spherical particles [11]. Surface chemistry is also known to impact the in vivo response to the injected materials [12–15]. For example, polyurethane functionalized with zwitterionic or anionic functional groups showed lower in vivo inflammatory reaction than those functionalized with cationic chemistries [16]. Similarly, polypropylene particles coated with carboxylic acid groups were less inflammatory than those coated with amine or hydroxyl groups [14].

Particle characteristics also influence the choice of in vivo application of the filler. For example, the stiffness of a filler needs to be different depending upon its injection location, functional

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requirements and desired residence time. Fillers used to treat urinary incontinence need to be stiff to impart strength, while facial fillers need to be relatively soft. Hyaluronic acid facial fillers with a lower degree of crosslinking, which are therefore softer, have a shorter residence time [17]. Further softer facial fillers are used in superficial injections around the lips and eyes, while stiffer fillers are used to fill deeper wrinkles [18–20]. Also, in the cases of Perlane® and Restylane®, both manufactured by the same company, the former contains larger particulates and is used to fill deeper wrinkles, while Restylane®, with smaller particulates, is suggested for use on superficial wrinkles [19].

The size, morphology, stiffness and surface chemistry of biomaterial particulates contribute to their in vivo application and performance. However, current manufacturing technology platforms offer limited independent control of particle size, shape and surface chemistry. Hyaluronic acid (HA) based soft fillers are made using top-down methods involving bulk polymerization, followed by screening and homogenization to break down the bulk gel into irregularly shaped, polydisperse particles [6,18]. For example, Radiesse VoiceGel® and Restylane® particulates are of irregular shapes and sizes. Other injectable fillers, such as Zyderm® and Cymetra®, result in particulates of irregular shapes and polydisperse sizes upon injection through a needle. Some fillers, such as Radiesse® and Deflux®, contain polydisperse yet controlled-size spherical microparticles (MP) of hydroxylapatite or dextranomers to impart stiffness.

Current methods to synthesize hydrogel MP of controlled size and shape include microfabrication-based techniques [21–24]. While microfabrication techniques provide excellent control over size and shape, the methods are complex and "intrinsically more expensive", and have low throughput and yield [21]. Furthermore, the use of low molecular weight (MW) polymers at high concentrations to achieve high conversions often result in MP with relatively high stiffness [25,26]. Developing methods that allow preparation of sufficient quantities of injectable fillers, especially with characteristics suitable for use as soft-tissue filler and independent control over its size, mechanical properties and surface chemistry, is desirable.

Here we report a suspension–polymerization-based method to create spherical, controlled-size hydrogel MP with tunable size and stiffness using photopolymerization. While some suspension photopolymerization-based methods have been reported earlier to prepare spherical microparticles for drug delivery [27–29], use of this method to prepare soft MP with tunable properties for use as injectable fillers remains relatively unexplored. Polyethylene glycol (PEG) was chosen to synthesize the MP because of its extensive use in FDA-approved or cleared products and its use in preparing biocompatible materials. HA and gelatin were chosen because of their favorable biocompatibility profiles, history of use in commercial dermal fillers and use in preparing soft, viscoelastic hydrogels [17]. Another motivation for using HA and gelatin to prepare MP was to demonstrate the generality of our method. The semi-interpenetrating technology (semi-IPN), which involves crosslinking of a polymer in the presence of a non-crosslinked polymer, was used to prepare the MP because this technology allows the preparation of materials with tunable properties by varying parameters such as the polymers used, their relative MW and their concentrations. Photocrosslinking was chosen because it can be carried out in ambient conditions, is efficient and affords precise spatiotemporal control over the crosslinking. The size distribution of suspension-polymerized MP can change as the reaction proceeds and, unlike chemical or thermal crosslinking, light-based crosslinking provides better control over the termination of the reaction [30]. Irgacure 2959 (I2959) was chosen as the photoinitiator (PI) because of its better cytocompatibility relative to other commercially available photoinitiators and its extensive use in preparing biomaterials, including those that encapsulate mammalian cells [31–34]. Furthermore, PEG hydrogels prepared using PEG diacrylate (PEG-DA) and I2959 were shown to be safe in humans in a 15-patient clinical trial for articular cartilage repair, thereby making the use of this system attractive [35]. In addition, GelrinC, a CE Mark-approved product in Europe, is also an in situ polymerized PEG-DA and I2959-based hydrogel that was shown to be safe and effective for the treatment of articular cartilage in injured knees over a period of 24 months in a multi-center clinical trial in Europe [36].

Semi-IPN MP of PEG-DA and PEG were prepared using suspension photopolymerization. The effect of process parameters such as stirring speed, surfactant concentration, gelation time, PI concentration and UV intensity on MP size and yield was investigated. In vitro cytocompatibility of the PEG MP was evaluated using the MTT assay. MP were also prepared using acrylated versions of HA and gelatin. The feasibility of preparing functionalizable MP and MP that encapsulate drug-releasing particles was also explored.

2. Materials and methods

2.1. Materials

PEG and PEG-DA (MW of both: 10 kDa) were purchased from Alfa Aesar (Lancaster, UK) and SunBio (Anyang City, South Korea), respectively. Dioctyl sulfosuccinate (AOT, sodium salt, 99%), n-hexane, PEG bis(amine) (MW: 3400 Da), trinitrobenzenesulfonic acid (TNBS), sodium dodecyl sulfate (SDS), sodium bicarbonate, hydrochloric acid, dexamethasone, polyvinyl alcohol (PVA), Rhodamine 6G, gelatin (300 g bloom strength, Type A from porcine skin) and methacrylic anhydride were from Sigma–Aldrich (St. Louis, MO). Sodium hyaluronate (MW: 351–600 kDa) and 2-hydroxy-1-[4-(2-hyroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959, PI) were from Lifecore Biomedical (Chaska, MN) and Ciba (Tarrytown, NY), respectively. Sulfosuccinimidyl acetate (sulfo-NHS-acetate) was from Thermo Scientific (Rockford, IL). Polyoxyethylene (20) Sorbitan Monoeleate (Tween 80) was from VWR (West Chester, PA). Ester-terminated 75:25 poly(DL-lactide-co-glycolide) (PLGA; inherent viscosity: 0.55–0.75 dl g^{-1}) was from Durect Corporation (Cupertino, CA). India ink was from Becton, Dickinson and Company (Cockeysville, MD). The MTT cell proliferation assay kit and NIH/3T3 fibroblast cells were from American Type Culture Collection (Mannassas, VA). Cell culture media, serum, phosphate-buffered saline (PBS), and trypsin were from Invitrogen (Carlsbad, CA). Radiesse Voice Gel®, Restylane® and Zyderm® were from Merz Aesthetics (San Mateo, CA), Medicis Aesthetics (Scottsdale, AZ) and Allergan (Irvine, CA), respectively. All chemicals were used as received.

2.2. Preparation of PEG hydrogel MP

Solutions of PEG and PEG-DA (100 mg ml^{-1} each) in deionized (DI) water were mixed in a pre-determined volumetric ratio. PI was added to this solution (0.5 mg ml^{-1} final concentration) to prepare the precursor solution (1 ml), which was then added to an AOT solution in hexane (9 ml) in a 2 inch wide polypropylene beaker. This suspension was then stirred at a predetermined speed using a 1 inch long stir bar on an IKA RET basic ETS-D5 magnetic stir plate. While being stirred, the suspension was photopolymerized at 72 mW cm^{-2} (measured at 365 nm at the level of the base of the beaker) with an Omnicure Series 2000 UV lamp for 260 s. Unless otherwise noted, the AOT concentration was 2 mM and the stirring speed was 800 rpm. Post-polymerization, the suspension was centrifuged at 150 rcf for 2 min and the organic supernatant was removed. The resulting MP were washed three times, once with 0.1 vol.% Tween-80 and twice with DI water using