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The controlled release of drugs from emulsified, sol gel processed silica microspheres

Shula Radin*, Tiffany Chen, Paul Ducheyne

Center of Bioactive Materials and Tissue Engineering, Department of Bioengineering, University of Pennsylvania, 210 S. 33rd Street, Philadelphia, PA 19104, USA

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ABSTRACT

Controlled release silica sol gels are room temperature processed, porous, resorbable materials with generally good compatibility. Many molecules including drugs, proteins and growth factors can be released from sol gels and the quantity and duration of the release can vary widely. Processing parameters render these release properties exquisitely versatile. The synthesis of controlled release sol gels typically includes acid catalyzed hydrolysis to form a sol with the molecules included. This is then followed by casting, aging and drying. Additional steps such as grinding and sieving are required to produce sol gel granules of a desirable size. In this study, we focus on the synthesis of sol gel microspheres by using a novel process with only two steps. The novelty is related to acid–base catalysis of the sol prior to emulsification. Sol gel microspheres containing either vancomycin (antibiotic) or bupivacaine (analgesic) were successfully synthesized using this method. Both drugs showed controlled, load dependent and time dependent release from the microspheres. The *in vitro* release properties of sol gel microspheres were remarkably different from those of sol gel granules produced by grinding and sieving. In contrast to a fast, short-term release from granules, the release from microspheres was slower and of longer duration. In addition, the degradation rate of microspheres was significantly slower than that of the granules. Using various mathematical models, the data reveal that the release from sol gel powder is governed by two distinct phases of release. In addition, the release from emulsified microspheres is delayed, a finding that can be attributed to differences in surface properties of the particles produced by emulsification and those produced by casting and grinding. The presented results represent an excellent data set for designing and implementing preclinical studies.

1. Introduction

Controlled release focuses on delivering biologically active agents locally over extended periods of time [1–3]. The site specificity of the delivery reduces the potential side effects that can be associated with general administration of drugs through oral or parenteral therapy [1]. Prevalent mechanisms for the delivery of biological agents by controlled release devices are resorption of the drug carrier material and diffusion. The resorption of these devices may, however, cause an inflammatory tissue response, which interferes with the treatment sought for with the molecules [4,5]. Thus, excellent controlled release materials are ideally biodegradable materials with generally good biocompatibility.

Room temperature processed, silica based sol gels are resorbable materials with a favorable tissue response [2,9]. They have been studied for biomedical applications that include tissue, cell and enzyme encapsulation and controlled release of drugs [2,3,6–13]. Derived from a metal alkoxide precursor, the sol is produced through a hydrolysis and polycondensation reaction [14]. Due to the mild processing conditions, high concentrations of many types of biologically active agents can be incorporated in the liquid sol. The agents are embedded in the matrix of the gel, which after condensation and drying becomes a porous, glassy solid [2,3,9–13]. Data show that controlled release of antibiotics, proteins, and growth factors is possible from this porous material [2,9–13]. These studies also demonstrate that the release is dependent on synthesis parameters such as the molar ratio of silica precursor to water, type of precursor and the concentration of bioactive drugs [2,10,11].

Controlled release sol gels are usually manufactured through an acid catalyzed process followed by casting, aging and drying. This leads to the synthesis of pellets, which can then be ground and sieved to arrive at granules or powders [2,3,10–12].

Sol gel granules made by grinding down cast discs possess an angular geometry. The sharp edges of this geometry may elicit more of an inflammatory response than that expected from microspheres. So far microspheres have mostly been made with

* Corresponding author. Tel.: +1 215 898 5140; fax: +1 215 573 2071.
E-mail address: radin@seas.upenn.edu (S. Radin).

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biodegradable polymers such as polylactic acid, polyglycolic acids and polylactic-co-glycolic acid [15,16]. These microspheres, however, are not ideal as their degradation products have been observed to cause an inflammatory response [4,5]. This probably would not be the case for sol gel microspheres, since it has been shown previously that silica sol gel granules demonstrate a favorable tissue response and enhanced bone healing [2,9].

Sol gel microparticles have been synthesized using spray drying [17] or emulsification [6,18]. The spray dried microspheres have been used in controlled release studies, however, these particles do not have excellent release properties as the spray drying caused a major reduction of the surface area and resulted in transforming highly porous sol gels into a dense material [17].

In this study, we focus on obtaining porous, controlled release sol gel microspheres using emulsification as the synthesis route. These microspheres were made both with and without biological agents incorporated. Specifically, we incorporated the antibiotic vancomycin and the analgesic bupivacaine. The selection of these molecules was related to our parallel programs that focus on osteomyelitis treatment [19] and surgical pain control [20]. Herein, we report on the synthesis parameters of emulsified acid – base catalyzed microspheres that affect optimal controlled release, with optimization being related to achieving release kinetics of vancomycin and bupivacaine with a desirable therapeutic profile. A novel acid–base catalysis was selected in order to shorten the time to gelation of the sol. A shorter time to gelation is essential to produce sol gel microspheres by emulsification. Synthesis parameters of interest were: pH and time to gelation of the acid–base catalyzed sol, water to alkoxide ratio in the sol, drug concentration added to the sol and rotational speed of emulsification.

2. Materials and methods

Sol gel derived silica microspheres were synthesized using an acid–base catalyzed hydrolysis of tetraethoxysilane (TEOS, Strem Chemicals, Newburyport, MA) followed by emulsification.

2.1. Typical sol synthesis

TEOS (10 ml) and 0.1 M HCl (2.4 ml), with and without the addition of de-ionized water (DI), were mixed and stirred to form an acid catalyzed sol. The molar ratio, R, of total water (including water in 0.1 M HCl) to TEOS varied from 2.5 to 10. Pharmaceutical agents were then added to the sol. Sols with 20 mg/g and 30 mg/g of vancomycin (drug to SiO_2 ratio), and sols with 50 mg/g and 80 mg/g of bupivacaine were made by adding corresponding amounts of the drugs. Prior to base addition, the acid catalyzed sol was cooled down to 4 °C in an ice bath. Subsequently, 0.08 M NH_4OH was added dropwise to the sol, which was thoroughly stirred. This changed the process to an acid–base catalyzed process. Depending on the amount of base added (2.2–2.4 ml), the pH of the sol was between 4.5 and 6; under these conditions, the time to gelation varied from immediate gelation to 1 h. To produce microspheres with and without the drugs, the pH was ideally set to 5.5, which led to a time to gelation between 15 and 40 min. Typically, 5 ml of the sol was applied dropwise onto 100 ml of vegetable oil stirred at speeds between 220 and 880 rpm by using a 2 inch × 3/8 inch magnetic stirrer. The stirring continued until microspheres precipitated to the bottom of the beaker. The microspheres were filtered through a 40 μm nylon filter, rinsed with DI water and left to dry overnight in a laminar flow hood.

2.2. Addition of biological molecules – variation of the ratio R

Vancomycin (vancomycin–HCl; Abbott Labs, Chicago, IL), as a first molecule, was dissolved in DI water at 100 mg/ml for incorporation into the sols. Bupivacaine (Spectrum, New Brunswick, NJ), the other molecule, was dissolved in methanol at 70 mg/ml for incorporation into the sols. The vancomycin and bupivacaine solutions were added to the acid catalyzed liquid sol to achieve calculated drug concentrations (mg of drug per gram of dried silica) of 20 and 30 mg/g of vancomycin and 50 mg/g of bupivacaine.

The water content of the sol was found to be critical in obtaining a clear sol without the precipitation of the molecules. The effect of water content on the incorporation of vancomycin into the sol was studied by using acid catalyzed sols without any DI water added to the sol (taking into account the presence of H_20 in the acid, R is equal to 2.75) or sols with total water/TEOS molar ratios (R) of 5, 6, 8, and 10. As shown in Table 1, acid catalyzed sols without extra water added to the sol became cloudy upon drug addition, indicating precipitation of the drug. When water was added to reach $R = 4$, low doses of vancomycin (16.7 mg/g) could be added to the acid catalyzed sol. However, precipitation of vancomycin was still seen when base was added. At R equal to 5, low doses (doses up to 20 mg/g) were successfully incorporated: no precipitation was observed after the addition of the drug and base. It must be pointed out, though, that after incorporation of the base, vancomycin precipitation was observed at higher doses such as 28 mg/g. Only at total water/TEOS ratios of 8 and above was the higher load successfully incorporated. This suggests that, in contrast to the sol gel synthesis with low water content as described by others [6], incorporation of these drugs requires the presence of a sufficient amount of water with R values greater than 5.

The addition of pharmaceutical agents and the variation in R also altered the pH and time to gelation of the sol. The volume of base was modified to maintain the time to gelation within the optimal range of 15–40 min.

2.3. Materials characterization

Morphology and size distribution of the microspheres were determined microscopically using an image analysis system consisting of a high resolution video camera and Image-Pro Plus 4.0 analysis software (Media Cybernetics, Silver Spring, MD). Sieving was also used to determine the size distribution. Nylon microporous filters of 70, 105, 210, 350, 500, and 710 μm were used. In addition, scanning electron microscopy (SEM, JEOL-6400) was used for imaging the morphology of microspheres in the size range below 100 μm.

Porosity of acid–base catalyzed ground granules and emulsified microspheres was measured using gas (nitrogen) sorption analysis (Autosorb 1, Quantachrome). Granules and microspheres of the same R8-30 V composition were used for the analysis (they contained 30 mg/g vancomycin by weight and were synthesized with water/TEOS ratio of 8). Prior to the analysis, the samples were outgassed at 50 °C for 24 h. Adsorption–desorption isotherms and multipoint BET [28] were used to determine porosity characteristics such as the specific surface area, pore volume, pore size distribution and the average pore size.

2.4. In vitro release and degradation study

In vitro release and degradation properties of microspheres were studied in phosphate buffered saline (PBS, Gibco, pH = 7.4) at 37 °C in comparison to those of granules. The size of particles was between 210 and 500 μm. As was the case for the microspheres, the ground granules were also produced from acid–base catalyzed sols. 1 ml of the sols was cast into vials, aged for 3 days and dried at room temperature until there was no further weight loss. The resulting sol gel discs were ground and then sieved to produce granules of the right size range.

For the release studies, 25 mg of particles were immersed in 5 ml of solution (5 mg/ml) and the solutions were exchanged daily. The dissolution experiments were conducted differently. In fact, in order to prevent solution saturation with silicon, 5 mg of particles were immersed in 5 ml of solution (1 mg/ml) and the solutions were exchanged at 6, 10, 24 and 48 h.

The concentration of drug released into solution was measured every 24 h. A time zero measurement was not included, as desorption phenomena are not typically observed with sol gel particles.

Table 1
The effects of water/TEOS molar ratios (R) and vancomycin load (drug to SiO_2 ratio in weight %) on the incorporation of vancomycin into acid catalyzed (AC) and acid–base catalyzed (ABC) sols.

Vancomycin loading	Water to TEOS molar ratio (R)								
	DI water-free	4		5		8		10	
	AC sol	AC	ABC	AC	ABC	AC	ABC	AC	ABC
16.7 mg/g	Cloudy	Clear	Cloudy	Clear	Clear	–	–	–	–
22.2 mg/g	–	–	–	Clear	Clear	Clear	Clear	Clear	Clear
28 mg/g	–	–	–	Clear	Cloudy	–	–	–	–
33 mg/g	–	–	–	–	–	Clear	Clear	–	–