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# Ultrasonic Welding Method to Fabricate Polymer Microstructure Encapsulating Protein with Minimum Damage

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## Introduction

Biodegradable polymers, including poly-lactic acid, polyglycolic acid, and their copolymers, are suitable biomaterials for drug delivery and implants because they are biocompatible and can be safely broken down into monomers by hydrolysis.[1-3] Recently these biodegradable polymers have been used in biological microdevices for drug delivery as well as in biosensors and tissue substitutes.[1,3-7] The interaction of biodegradable polymers with cells has been investigated in the development of cell-based biosensors for diagnostic analysis of drugs, pathogens, and toxicants.[8,9] Biodegradable polymers are suitable as cell-based biosensors because they are able to integrate cells into transducers by providing an artificial scaffold. In addition to framework function, the sustained release property of biodegradable polymers has been exploited to accelerate cell growth through the release of growth factor from the structure.[10]

Previous scaffold structures have been fabricated by porogen-based methods which typically involve porogens within the polymer matrix that are subsequently removed to leave behind porous voids.[11,12] However, these methods are not suitable for grafting into microstructures because this approach is limited to microdevice geometries that permit complete porogen removal. The microstructure for tissue engineering research requires more sophisticated geometry in addition to porous structures to control cell growth.[13]

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Microscaffolds have been prepared by micropatterning and micromolding.[13-15] Micromolding techniques have been developed to fabricate microstructures with low cost, simple process, and the potential for mass production by injection molding and embossing instead of micropatterning. However, previous micromolding methods were based on injection molding. This process requires high temperature and high pressure, resulting in the denaturation of proteins and thermally sensitive drugs.[16] Thus, it is difficult to encapsulate thermally sensitive compounds inside of a polymeric microstructure.[17] For sophisticated polymeric microstructures that encapsulate biological compounds, a new process is needed for microlevel cell culturing and delivery of the compound into the cells. To minimize thermal damage, the material must be handled at low temperatures, with exposure to high temperatures limited to the shortest possible duration.

Microparticles and nanoparticles, which have a flow property like that of fluids, can be filled into molds and then ultrasonically welded to make polymeric medical devices. Ultrasonic welding is the fastest available welding technique, with weld times of less than few seconds. With this method, the polymer molecules are mechanically vibrated at high frequencies to form bonds at the interfaces of the microparticles. The interfacial bonding is primarily generated by the transfer of energy produced by the vibrating molecules of the polymer.[18] This process enables fabrication of polymeric microstructures with less damage to the encapsulated material than would result from high-temperature processes.

We fabricated polymeric structures encapsulating thermally sensitive compounds by filling poly-di-methyl-siloxane (PDMS) molds with poly(lactic-*co*-glycolic)acid (PLGA) microparticles encapsulating bovine serum albumin (BSA) and Vitamin B. The polymer microparticles were prepared by means of a spray drying method and used to fabricate polymer microstructures by means of ultrasonic welding. Potential morphologic changes in structure were investigated with scanning electron microscopy (SEM). The stability of BSA in polymer structures was investigated in terms of the amount of applied energy using light scattering and protein solubility methods to measure any damage to BSA resulting from the ultrasonic welding process.

## Experimental

**Preparation of Polymer Microparticles.** PLGA particles encapsulating Vitamin B were prepared by spray drying (Buchi 290 Mini Spray Dryer, Flawil, Switzerland). Five mL of a 3% Vitamin B (Fiboflavin-5'-phosphate sodium salt dehydrate, Avocado Organics, Lancashire, England) solution was emulsified in 100 mL of a 3% (w/w) PLGA (LACTEL,

inherent viscosity 0.9-1.2 dL/g) solution in ethylacetate (Sigma-Aldrich, St. Louis, MO) by using a sonicator (Sonics & Materials, Vibra Cell, Danbury, CT). The emulsion solution was then spray dried. The inlet and outlet temperatures of the spray dryer were 60 °C and 40-45 °C, respectively. The BSA-loaded microparticles were also prepared by using the spray-drying method. The oil phase was prepared by dissolving 6 g of PLGA 50/50 in 24 g of methylene chloride (Aldrich). The water phase was a 10% BSA (Sigma) solution in phosphate-buffered saline (PBS). One mL of BSA solution was added to 10 mL of PLGA solution and then sonicated for 20 s with the sonicator. The resultant oil-in-water emulsion was spray dried to produce microparticles at an inlet temperature of 55 °C and an outlet temperature or 40 °C.

Particle size was measured by using a Beckman Coulter Multisizer (Beckman Coulter, Fullerton, CA).

**Preparation of Polymeric Scaffolds from Microparticles Using Ultrasonic Welding.** The ultrasonic welding process is summarized in Figure 1. For BSA, a Teflon mold with a cylindrical well 3 mm in diameter and 1 mm in depth was used for preparation of the polymeric scaffolds. The mold was covered with 300 mg of PLGA microparticles containing BSA, and then the microparticles were pushed into the well. Next, a 1-mm thick PDMS layer was put on top of the PLGA particle cake to prevent the sticking of particles to the ultrasonic tip, and the ultrasonic horn was applied on the PDMS layer with a force pressure of 2 kg. Next, the polymer particles were welded by ultrasonication at different exposures (22% power and 500 W maximum output) using a 20 kHz ultrasonic device (Sonics & Materials) with a 1 s operation pulse.

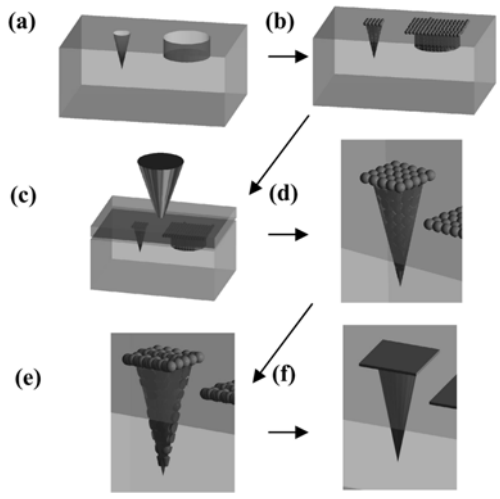


**Figure 1.** Ultrasonic welding of microparticles encapsulating thermally sensitive compounds to fabricate three-dimensional microstructures: (a) mold preparation; (b) placement of microparticles encapsulating compounds on mold; (c) welding at interface between microparticles; (d) interfacial bonding of microparticles; (e) dense interconnecting of microparticles; (f) bulk melting of microparticles to form solid microstructures.

The PLGA microparticles encapsulating Vitamin B were filled into PDMS molds with tapered pyramid-shaped wells. Then the microparticles were ultrasonically welded in the micromold in the same manner as for BSA.

**Morphology.** The effect of ultrasonic welding on morphology of the final structure was investigated by using SEM (Hitachi 3500H, Tokyo, Japan) with increasing periods of applied energy (10 s, 20 s, 40 s, and 80 s at 110 W) after gold coating using a sputter coater (Ernest F. Fullam, Latham, NY).

**Protein Stability.** To assess possible protein damage caused by ultrasonic welding, two different assays were conducted to investigate protein stability of BSA after ultrasonic welding. In the first assay, after treatment, the PLGA structures were dissolved in acetonitrile, and the BSA particles were recovered by filtration and dried. The BSA samples were then dissolved in phosphate buffer saline (PBS) to measure the concentration of BSA remaining soluble in PBS. Insoluble aggregates were separated by centrifugation at 30,000 g for 30 min. BSA concentration in the supernatant was determined by the Lowry assay.[19] In the second assay, the presence of soluble aggregates of BSA was detected by dynamic light scattering.[20] BSA particles were dissolved in PBS at a concentration of 2.5 mg/mL, and 3 mL of BSA solution in PBS was placed in a quartz cuvette at room temperature for dynamic light scattering measurements (Brookhaven, Holtsville, NY) using CONTIN analysis.

## Results and Discussion

**Fabrication of Microparticles.** The PLGA microparticles ranged from 1 to 7 $\mu$m in size. After fast-drying in the drying chamber of the spray dryer, the microparticles had a corrugated surface (Figure 2(a)), and their particle size was monodisperse. The average particle size was $3 \pm 5$ $\mu$m.

**Effect of Ultrasonic Welding on Microstructure.** During ultrasonic welding with 2 kg of applied force, SEM showed that the morphology of the microstructure changed from a loosely connected network to a solid structure (Figures 2(b)-(e)). During phase I (Figure 2(b)), the PLGA microparticles bonded at the interface of the microparticles with numerous pores inside the structure. Comparing the volume with the weight of the structure, during this phase the porosity of the BSA-containing microparticles in the 3-mm × 1-mm well was calculated to be 85%. With increasing duration of energy application, the porous structure became more packed and the interfacial bonding area between microparticles increased. During phase II, the microparticles still had their original shape but had started to merge together into a fused structure (Figure 2(c)). The porosity of the structure in phase II was 75%. During phase III, the porous structure became more dense (Figure 2(d)). At this stage it was difficult to find the original shape of microparticles. Melting had

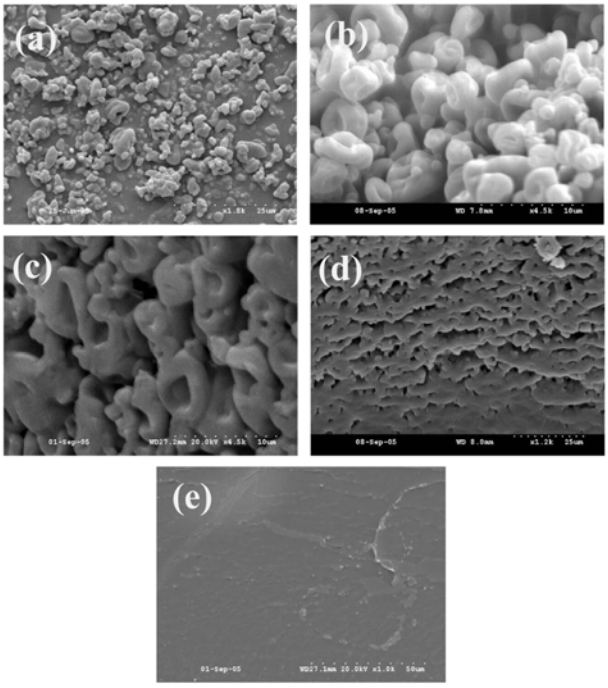


**Figure 2.** SEM photography of morphologic changes in structure of microparticles encapsulating BSA during ultrasonic welding at increasing duration of application of ultrasonic energy under 2 kg of force: (a) spray-dried microparticles; (b) phase I, loosely connected microparticles with ultrasonic welding between microparticles; (c) phase II, densely connected microparticles; (d) phase III, porous structure; (e) phase IV, solid structure.

propagated through the microparticles, in addition to the interface between them, but empty spaces could still be observed. Finally, in phase IV, all microparticles had melted and the empty spaces had been removed by applied pressure, resulting in a solid structure (Figure 2(e)).

The changes in morphology during these four phases corresponded to increased duration of energy application. Thus, phase of structure can be selected as needed. To make a porous structure for cell culture, phase II of III may be preferred, whereas for solid microstructures requiring mechanical support function, phase IV may be selected.

To investigate the ability of ultrasonic welding to copy high aspect ratio structures, a tapered pyramid mold was used to copy structure. Figure 3(a) shows a microscopic image of a portion of an array of pyramid structures (600 $\mu$m in height and 250 $\mu$m in base diameter) made from microparticles encapsulating Vitamin B which has fluorescent property. The base layer was made of polystyrene involving ultrasonic welding of white polystyrene film to the bases of the tapered pyramid structures. The total time to make the solid structures was 80 s at 100 W power, a very short time compared to conventional micromolding processes using high temperature and high pressure. After 5 s, due to the relatively low power level (100 W) used in this experiment to

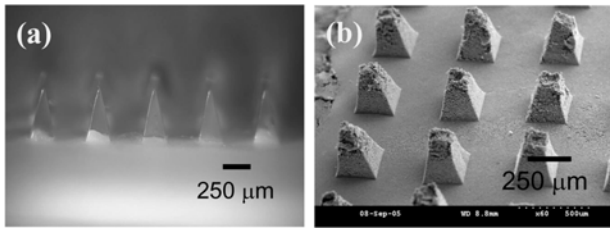


**Figure 3.** Optical microscopic images of solid tapered pyramid-shaped structures made through local transfer of ultrasonic energy into a mold containing microparticles encapsulating Vitamin B: (a) fully solidified microstructure; (b) partially solidified microstructure.

achieve a sophisticated level of power control compared to the high power of commercialized ultrasonic welding devices, the microparticles in the tip of the mold had not completely melted (Figure 3(b)). The ultrasonic energy had not yet fully transferred to the microparticles.

**Protein Stability.** The stability of proteins in polymeric structures made by ultrasonic welding is our main interest. We selected BSA as a model drug to investigate changes in stability because the thermal stability of BSA has been thoroughly studied, and BSA is included in cell culture medium to improve cell survival. The aggregation of insoluble protein due to irreversible denaturation was assessed by measuring aqueous BSA solubility after ultrasonic treatment. Exposure to ultrasonic energy for 10 s, 20 s or 40 s, and 80 s lowered protein solubility by 1.1%, 3%, 5.6% and 10%, respectively. It typically takes less than 1 min to make solid polymeric structures of encapsulated BSA by using ultrasonic welding with 100 W of power. Thus, we can make solid structures with minimum damage to proteins encapsulated in the microstructure for tissue substitute and sustained release (Figure 4(a)). A solid microstructure in which nanoparticles or microparticles are dispersed monolithically can be fabricated by this method.

To further test stability, re-dissolved BSA in PBS was assayed using dynamic light scattering (DLS). As a type of analysis, an inspection of the samples in solution is provided by DLS measurements. Results are shown in Figure 4(b) together with those obtained for native protein. A cumulative analysis of DLS data gives an average size of $4.1 \pm 0.1$ nm, $4.24 \pm 0.39$ nm, $4.5 \pm 0.3$ nm, $4.5 \pm 0.6$ nm and $5.1 \pm 1.5$ nm after 0 s, 10 s, 20 s, 40 s and 80 s of ultrasonic energy exposure respectively. The size distribution of the sample exposed for 80 s suggests that, during ultrasonic welding, conformational changes in BSA partially occur through melting of polymer microparticles due to thermal exposure. However, 80 s of exposure is a harsh condition, and ultrasonic welding is usually capable of fabricating solid structures within less than 80 s. Thus, almost all of the BSA in polymeric structures would keep its inherent properties after ultrasonic treatment under industrial sonication conditions with expo-

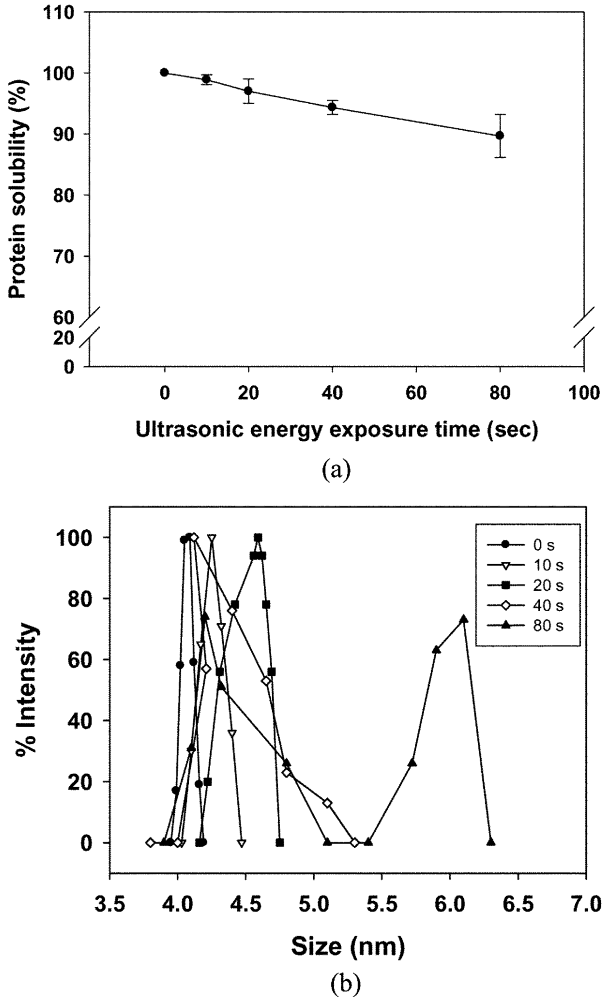


**Figure 4.** Stability of bovine serum albumin (BSA) after treatments: (a) BSA solubility in water with increasing length of exposure to ultrasonic energy; (b) size distribution change of BSA by exposure of BSA to ultrasonic welding under 2 kg force.

sure for only a few seconds at high power levels.

## Conclusions

Ultrasonic welding can be used with micromolding to fabricate polymeric microstructures. We investigated whether these techniques are useful in fabricating polymeric microstructures from polymeric microparticles that encapsulate thermally sensitive compounds. We used a spray-drying method to prepare biodegradable polymer microparticles encapsulating the thermally sensitive compound, BSA and Vitamin B. The resulting polymeric structures were less than 7 $\mu$m in diameter. Morphological changes after increasing periods of application of ultrasonic energy could be categorized into four phases: loose connecting, dense connecting, porous packing, and solid structures. The stability of polymer structures encapsulating BSA was tested after increasing periods of applied energy. Few changes were seen after typical periods (under 1 min), but 10 % of BSA was denatured after 80 s (considered to be harsh conditions). More work is needed to optimize this novel method of microfabrication for delivery of other heat-sensitive protein and growth factors and the biological function of protein also need to be investigated in next experiment. However, it appears to a promising means of fabricating polymer microstructures for cell chips, tissue engineering, and drug delivery, in which protein must be encapsulated with minimal denaturation.

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