PLGA Micro and Nanoparticles Loaded into Gelatin Scaffold for Controlled Drug Release

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Abstract—Curcumin and bovine serum albumin (BSA) were used as model drugs and loaded into micro and nanoparticles of biodegradable poly(lactic-co-glycolic acid) (PLGA). The PLGA was incorporated into hydrophilic and biocompatible gelatin scaffolds to design a controlled drug release system. The gelatin scaffolds were cross-linked using glutaraldehyde. The controlled delivery of drugs from biologically active PLGA micro and nanoparticles was measured and showed consistent release for 30 days. Curcumin and BSA loaded PLGA micro/nanoparticles based gelatin scaffolds show a novel approach to embed multiple drug molecules to overcome multidrug resistance as well as depict a new type of biocompatible and biodegradable implants. Such scaffold constructs can be used for breast implants after lumpectomy to not only overcome cosmetic issues but also to provide constant drug release during healing process. In one type of construct, only BSA loaded microparticles were mixed with gelatin, while in the other type of construct, both BSA and curcumin loaded PLGA microparticles were embedded. BSA and curcumin loaded nanoparticles were also embedded in gelatin constructs to see the effects of particle size on drug release. After 30 days, cumulative BSA release from PLGA micro and nanoparticles embedded in gelatin scaffold were measured to be 69.87% and 86.11%. The cumulative release of curcumin was measured to be 53.11% and 60.42% from curcumin loaded PLGA micro and nanoparticles, respectively. A statistically significant difference was seen in cumulative drug release from these scaffolds (p-value < 0.05).

Index Terms— Gelatin scaffold constructs, Controlled drug delivery, Cross-linker, Multi-drug resistance, BSA, Curcumin.

I. INTRODUCTION

Breast tumor patients are often treated with surgical interventions followed by radiotherapy [1]. Lumpectomy (surgical removal of tumor tissues) leaves hollow cavities which pose cosmetic concerns. The plastic surgery might help to some extent, but it is very expensive [2-4]. Regular radiation therapy is also expensive, time consuming, non-specific, and increases the cosmetic deformity of surgery [5, 6]. Therefore it is a challenge to develop a novel treatment for breast cancer to ensure improved cosmetic features and controlled drug delivery to eliminate tumor regeneration. Recent advances in biotechnology have made it possible to synthesize various scaffolds for drug delivery and tissue culture applications. Proteinous drugs are often used most of which are susceptible to degradation during storage and administration in a body [7].

Gelatin is a biodegradable, biocompatible, non-carcinogenic, non-immunogenic, and inexpensive natural polymer derived from a protein called collagen [8]. Inherent biocompatibility, uniformly distributed interconnected porosity, and appreciable mechanical strength have made gelatin scaffolds fascinating for drug delivery and tissue engineering applications. Moreover their hydrophilic, soft, and rubbery nature ensures minimal tissue irritation. Consequently, gelatin scaffolds have been used as a promising material in bone tissue engineering, hepatic tissue engineering, and artificial skin regeneration [9-12]. Since gelatin is soluble in aqueous solutions, gelatin-based drug delivery systems need to be cross-linked in order to impart longevity to scaffold so controlled drug release is achieved. Different aldehydes such as glutaraldehyde and formaldehyde are widely used cross-linkers for gelatin [8].

Over the past few decades, there has been increasing interest in developing biodegradable micro/nanoparticles for drug delivery applications. Such particles offer enhanced surface area making them very effective for targeted drug delivery [13-15]. Poly(lactic-co-glycolic acid) (PLGA) is a biocompatible and biodegradable polymer that is synthesized using ring opening polymerization of two monomers; glycolic acid and lactic acid. PLGA is widely used in tissue engineering applications with various ratios of lactic acid and glycolic acid [16, 17]. Further, PLGA microparticles can be prepared by single emulsion or double emulsion methods. Single emulsion is a simple method where only one water phase solution is cross-linked with oil phase solution. Water-in-oil-in-water double emulsion consists of dispersed oil globules containing smaller aqueous droplets. These materials
offer interesting possibilities for the controlled release of chemical species initially entrapped in the internal droplets.

Curcumin is a component of turmeric, which has been reported for its chemo-preventive and chemotherapeutic activity in a number of cancer types through influencing cell cycle arrest, differentiation, apoptosis, and so on [18, 19]. It was used as hydrophobic model of drug in our design. On the other hand, albumins are a group of acidic proteins, which occur abundantly in body fluids and tissues of mammals. Serum and plasma albumin contains 50% proteins. Bovine serum albumin (BSA), also called as ‘Fraction V,’ was used as hydrophilic drug model in our design.

Multidrug resistance (MDR) is a mechanism by which cancer cells develop resistance against therapeutic drugs [20-22]. Therefore more than one drug is recommended for chemotherapy to overcome MDR. In case of lumpectomy, a biodegradable scaffold construct that can not only mitigate the cosmetic effects of tissue removal but can also overcome MDR is ideal. The scaffold should also give sustained drug release over extended period of time. In one report, doxorubicin was loaded in PLGA micro and nanoparticles which were embedded in cross-linked gelatin scaffold [23]. The design was simple yet sustained drug release over a period of one month. Our goal was to improve the design to provide enhanced control on drug release profile along with the capability to overcome MDR. Our design consisted of four types of gelatin scaffolds i.e.

- **Scaffold_1:** Gelatin scaffold with BSA loaded PLGA microparticles embedded into it
- **Scaffold_2:** Gelatin scaffold with BSA and curcumin loaded PLGA microparticles embedded into it
- **Scaffold_3:** Gelatin scaffold with BSA loaded PLGA nanoparticles embedded into it
- **Scaffold_4:** Gelatin scaffold embedded with curcumin loaded PLGA nanoparticles

Throughout this article, we will use the terms Scaffold_1, Scaffold_2, Scaffold_3, and Scaffold_4 instead of their embedded types for simplicity.

### II. MATERIALS AND METHODS

#### A. Synthesis of BSA Loaded PLGA Microparticles

The materials used to prepare PLGA microparticles by double emulsion method (water-in-oil-in-water) included deionized (DI) water, dichloromethane (DCM), PLGA, polyvinyl alcohol (PVA) and BSA. A 1.5 ml centrifuge tube was used to dissolve 20.5 mg of BSA in 0.2 ml of DI water, to make the water phase of the solution ($W_1$). To make PLGA solution, 0.1 g of PLGA was dissolved in 1 ml of DCM in a glass tube. This formed the oil phase ($o$). A 25 ml glass flask was used to dissolve 0.2 g of PVA in 20 ml of warm DI water (50 °C) to make 1% aqueous PVA solution. This formed the second water phase solution ($W_2$). In order to form the microparticles, the drug solution was added to the PLGA solution and was vortexed for 30 seconds. This was “water-in-oil” phase. This solution was then added to the aqueous PVA solution thus forming water-in-oil-in-water ($W_1$-$in$-$o$-$in$-$W_2$). The resultant solution was vortexed again for 60 seconds to make microparticles. A stir bar was dropped in the flask and the mixture was gently stirred for 1 hour in a chemical hood allowing the DCM to evaporate and then this was allowed to stabilize for 45 minutes. Next, the solution was transferred into a 50 ml centrifuge tube and was centrifuged at 4000 rpm for 15 minutes. The supernatant was then collected and frozen for calculating the loading efficiency using an indirect method. The pellet was resuspended in 5 ml PBS and vortexed for 1 minute. The sizes of PLGA microparticles were measured from scanning electron microscope (SEM) micrographs. The stability of the microparticles was measured using Zeta Potential Analyzer (Sympatec, Germany). The resulting solution was freeze dried to get the microparticles. The loading efficiency was calculated using the formula,

$$\text{Loading Efficiency} \% = \frac{X - Y}{X} \times 100$$

Where $X$ was the original protein amount and $Y$ was the amount of protein in the supernatant.

#### B. Synthesis of Curcumin Loaded PLGA Microparticles

The materials used for making PLGA microparticles using single emulsion method included DI water, DCM, phosphate buffered saline (PBS), curcumin as a hydrophobic drug model, PVA and Ethanol. PLGA-drug solution was made by dissolving 20 mg of curcumin and 0.1 g of PLGA in 1 ml of DCM in a glass test tube (oil phase). Two hundred mg of PVA was dissolved in 20 ml of warm DI water (50 °C) to make an aqueous PVA solution which acted as water phase (1% PVA solution). The microparticles were emulsified by adding the oil phase solution to PVA water phase solution. The resultant (o/w) solution was vortexed for 60 seconds, gently stirred for one hour to evaporate all the DCM and then centrifuged for 15 minutes at 4000 rpm. The supernatant was then collected and freeze dried at −80 °C to calculate the loading efficiency using an indirect method. The pellet was resuspended in 3 ml of PBS solution. The PLGA particles were freeze dried and their sizes were measured from SEM images. The stability of the microparticles was measured using Zeta Potential Analyzer. The loading efficiency was calculated using equation (1).

#### C. Synthesis of BSA Loaded PLGA Nanoparticles

The BSA loaded PLGA nanoparticles were synthesized using double emulsion method. The method was same as that for BSA loaded microparticles synthesis except few changes i.e. 5% PVA solution was used instead of 1%. The drug and polymer mixture was sonicated at 30 W for 2 minutes to get the nanoparticles. Particles were collected by centrifugation at 10,000g for 15 minutes and the pellet was resuspended into 5 ml PBS and freeze dried. The nanoparticles were analyzed using SEM and the stability of the nanoparticles was measured using Zeta Potential Analyzer. The supernatant was also freeze dried and mixed with 10 ml of DI water to calculate the indirect loading efficiency using equation (1).

#### D. Synthesis of Curcumin Loaded PLGA Nanoparticles

The curcumin loaded PLGA nanoparticles were synthesized using single emulsion method. The method was same as that
used to synthesize curcumin loaded microparticles except few changes i.e. 5% PVA solution was used instead of 1%. Again drug and polymer mixture was sonicated at 30W for 2 minutes to get the nanoparticles. Particles were collected by centrifugation at 10,000g for 15 minutes and the pellet was resuspended into 3 ml PBS and freeze dried. The particles were analyzed using SEM and the stability of the nanoparticles was measured using Zeta Potential Analyzer. The supernatant was also freeze dried and mixed with 10 ml of DI water to calculate the indirect loading efficiency using the formula of equation (1).

E. Synthesis of Gelatin Scaffold and Embedding of Micro and Nanoparticles

The materials used for gelatin scaffold formation were gelatin, 1% glutaraldehyde solution, glycine and DI water. Using a glass beaker, 25 ml of 3% gelatin solution was stirred at 5000 rpm in warm DI water (65 °C) and then 1% glutaraldehyde solution was added followed by 37 °C stirring for 5 minutes. Freeze-dried particles were weighed and 4 mg of particles were added to gelatin during gelation. Solution was then poured into a well plate (mold) and kept overnight at room temperature. Then gelatin was washed with 0.1 M glycine followed by water wash (three times). Then gelatin scaffolds were frozen at −20 °C for 24 hours before drug release experiments.

F. Drug Release Studies

For drug release studies the amount of released drugs were measured up to 30 days at different time points using bicinchoninic acid (BCA) protein assays for BSA release following the manufacturer instructions (Pierce Biotechnology, Rockford, IL). The percent cumulative drug release was calculated from this data and temporal plots were created.

For curcumin release, after all the time points, 100 µl of each sample was homogenously mixed with 100 µl of ethanol. The drug release was quantified using 450 nm laser spectroscopy. The formula for calculating the % protein release was,

\[ \text{% Protein Release} = \frac{\text{Amount of released protein}}{\text{Amount of loaded protein}} \times 100 \] (2)

G. Scanning Electron Microscope (SEM) Analysis

For SEM analysis, PLGA micro and nanoparticles were attached to aluminum sample holder using double-sided carbon tape. To make samples conductive, 5 nm of gold was sputtered using Cressington Gold Sputtering Deposition Equipment. The samples were imaged with Hitachi S-3000N Variable Pressure SEM. Secondary electron detector was used at high vacuum for SEM imaging.

H. Statistical Analysis

Statistical comparisons between groups were carried out using analysis of variance (One-Way ANOVA). A difference was considered significant when \( p\text{-value} < 0.05 \).

III. RESULTS AND DISCUSSION

A. Sizes of PLGA Micro and Nanoparticles

The synthesized PLGA micro and nanoparticles were imaged with SEM. The morphology of PLGA particles were found to be spherical and smooth as shown in Figure 1. When sonicated, PLGA nanoparticles are known to form instead of microparticles [19]. In addition, we found that the higher PVA concentration and sonication yielded smaller PLGA nanoparticles. Laser scattering measured with Zeta Potential Analyzer was used to find the particle size distribution, polydispersity, zeta potential and average particle diameter. The average diameters for BSA loaded PLGA micro and nanoparticles were found to be 30.3 µm and 262.1 nm, respectively. For curcumin loaded PLGA micro/nanoparticles, the average diameters were 48.7 µm and 250.7 nm, respectively (Table 1).

Figure 1: SEM micrographs of PLGA micro and nanoparticles. (a) BSA loaded PLGA microparticles with average diameter of 30.3 µm. (b) BSA loaded PLGA nanoparticles with average diameter of 262.1 nm.
TABLE I

<table>
<thead>
<tr>
<th>Type</th>
<th>Mean Diameter</th>
<th>Polydispersity</th>
<th>Zeta Potential, $\zeta$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA loaded microparticles</td>
<td>30.3 µm</td>
<td>0.506</td>
<td>$-47.64 \pm 1.22$</td>
</tr>
<tr>
<td>Curcumin loaded microparticles</td>
<td>48.7 µm</td>
<td>0.696</td>
<td>$-42.04 \pm 4.19$</td>
</tr>
<tr>
<td>BSA loaded nanoparticles</td>
<td>262.1 nm</td>
<td>0.206</td>
<td>$-25.78 \pm 3.79$</td>
</tr>
<tr>
<td>Curcumin loaded nanoparticles</td>
<td>250.7 nm</td>
<td>0.419</td>
<td>$-21.17 \pm 3.04$</td>
</tr>
</tbody>
</table>

B. Particle Size Distribution

Particle size distribution is gauged from polydispersity index, a dimensionless number. Its value ranges from 0.01 (monodispersed particles) to 0.7 (particles with varying size distributions). Polydispersity index $> 0.7$ shows that particles have very broad size distribution [24]. The polydispersity of all types of PLGA particles is summarized in Table 1. Polydispersity index of all particles was found to be $< 0.7$ which showed that although the PLGA particles had wide size distributions (especially microparticles), but these were still in acceptable range. Overall, the PLGA microparticles gave higher polydispersity index values (0.506 and 0.696 for BSA and curcumin loaded microparticles, respectively) compared to the respective nanoparticles. The higher polydispersity index values for PLGA microparticles might have stemmed from the synthesis process. As microparticles were synthesized by uncontrolled vortexing for about 60 secs, it could have yielded particles with higher size distributions. The diameter distribution of PLGA particles is shown in bar graphs of Figure 2.

C. Colloidal Properties

The stability of the particles in solution is very important factor in any application that involves colloidal suspensions. The zeta potential, $\zeta$, commonly used to characterize the surface charge of the particles, is influenced by particle composition and the medium in which particles are dispersed. Particles with $\zeta > \pm 30$ mV are considered stable in the colloid suspension as surface charges prevent particle aggregation [25]. The zeta potentials for our PLGA micro and nanoparticles had negative values which suggested the presence of negative charges on the surfaces. PVA was used as surfactant during synthesis of PLGA particles which imparted negative surface charge due to physical entrapment within the layers of the PLGA polymer. The zeta potential of PLGA microparticles were found to be $> -30$ mV, which gave excellent colloidal properties. In the case of nanoparticles, although the zeta potential was $< -30$ mV, but it was good enough to have reasonably stable colloidal solution.

D. Drug Loading in Particles

The drug loading/encapsulation efficiency of PLGA particles is desired to be high so that less quantity of the particles would be required for certain drug dose administration. As BSA is degradable in organic solvents, indirect loading efficiency calculation method was used for all of the particles to determine loading efficiency. The loading
efficiencies for BSA loaded micro and nanoparticles were found to be 27.39% and 31.6%, respectively. The curcumin loaded PLGA micro and nanoparticles had loading efficiencies of 58.58% and 55.39%, respectively. Drug loading efficiency very much depends on the drug solubility in the scaffold matrix material, drug-polymer interaction, polymer composition and molecular weight of polymer [26-28]. As we have used the same PLGA polymer for synthesis of both of the micro and nanoparticles, we conclude that, herein, only the drug solubility and drug polymer interactions played role in drug encapsulation efficiency. Curcumin loaded particles gave more encapsulation efficiency as both of curcumin and PLGA polymers were hydrophobic in nature and curcumin was mixed thoroughly with PLGA polymer during synthesis (single emulsion). BSA showed lower encapsulation efficiency because of its hydrophilic nature.

Figure 3: SEM micrographs of PLGA microparticles embedded in gelatin scaffold (a) The black arrows indicate PLGA microparticles. The particles preserved their original shape after incorporation. (b) Magnified view of a few embedded PLGA microparticles.

In order to make embedded scaffolds, drug loaded particles were mixed into gelatin during gelation process as explained in the Materials and Methods section above. The gelatin scaffolds were imaged with SEM to verify the successful incorporation of drug loaded PLGA particles into scaffolds (Figure 3). Embedding of drug loaded PLGA nanoparticles into gelatin scaffold was verified using the same approach. The incorporated PLGA nanoparticles were not clearly visible during SEM imaging due to their smaller sizes.

E. Drug Release

In vitro drug release was monitored for 30 days. The cumulative BSA release was found to be 69.87% and 87.11% for Scaffold_1 and Scaffold_3 as shown in Figure 4(a). The more BSA release from Scaffold_3 was due to smaller size of embedded PLGA nanoparticles. Smaller PLGA nanoparticles had larger surface area and most of the loaded BSA would be present near the particle surface leading to fast BSA release. Whereas PLGA microparticles had larger cores leading to slow diffusion of BSA during release. For both scaffolds, however, abrupt release was seen for first 5 days followed by sustained drug release. There was significant statistical difference between the BSA release from Scaffold_1 and Scaffold_3 from day 5 to day 30 (p-value=0.034, n=4).

Comparing the BSA release form Scaffold_1 and Scaffold_2, it was observed that less BSA was released form Scaffold_2. The presence of curcumin along with BSA in Scaffold_2 had reduced the cumulative BSA release from Scaffold_2 (65.14%) as shown in Figure 4(b). However, the statistical difference between BSA release from Scaffold_1 and Scaffold_2 was not significant (p-value=0.431, n=4).

It was also noticed that more BSA was released compared to curcumin from the Scaffold_2. The cumulative curcumin release was found to be 53.11% compared to BSA release of 65.14% (Scaffod_2) over the period of 30 days. The hydrophobicity of curcumin may be the cause of its slow release as it might have produced localized hydrophobic environment on its release.

The curcumin release was also monitored for Scaffold 4 which had curcumin PLGA nanoparticles embedded into gelatin scaffold. It was observed that more of the BSA was released from Scaffold_3 (87.11%) compared to curcumin release from Scaffold_4 (60.42%) as shown in Figure 4(c). It further verified that the main cause of slow curcumin release was its hydrophobic nature, not the size of PLGA particles. It was reported recently that cumulative curcumin released from PLGA microparticles was almost 80% after 28 days (without incorporation into gelatin scaffolds) [29]. In our case, however, there was just 53.11% of curcumin released from PLGA microparticles embedded in gelatin scaffolds after 30 days. Our design gave significantly slow but sustained release as compared to the previous report.

To develop a good drug delivery system, both drug release and polymer degradation are important factors. The drug release mechanism from PLGA particles embedded into gelatin scaffolds is relatively complicated. It may comprise interactions like: (1) Water permeation through the gelatin scaffold resulting into gelatin swelling and degradation into smaller amino acid chains; (2) Water absorption by PLGA particles; (3) Degradation of PLGA particles by hydrolysis and break down of ester bonds; (4) Diffusion of BSA and curcumin through the degraded PLGA particles; (5) Diffusion of drug molecules through water filled tortuous paths in gelatin scaffolds.
There have been various reports of embedded polymer particles into different types of scaffolds. In one report, transforming growth factor, TGF-β1, was loaded into gelatin microparticles which were further incorporated into oligo(poly(ethylene glycol) fumarate) (OPF) hydrogels. More controlled release was observed when microspheres were incorporated into OPF [30]. In another report, Zhang et al. studied the release of hydroxyl functionalized glycerol poly(ε-caprolactone) in poly(n-isopropylacrylamide) (PNIPAAm) hydrogels [31]. Controlled release was reported for microparticles embedded into PNIPAAm hydrogels. However, single drug molecule release has the potential to develop MDR by cancer cells.

In the present work, the in vitro drug release from different types of scaffolds suggests its potential clinical utility for treatment of not only breast cancer patients but a variety of other malignancies which require sustained release over a long period of time. Our design has the capability to overcome MDR along with more control on drug release. Both of the drugs can be loaded into single PLGA microparticle i.e. BSA in first water phase, curcumin in oil phase. We expect that at the start, more curcumin would release because of its presence near the surface as compared to BSA which would be present inside the core. After few days, sustained released of BSA would be achieved. In this way, our design has inherent capability of scalability and much wider variety of possible modifications and drug choices as may be required.

IV. CONCLUSION

We have synthesized a controlled drug release system for BSA and curcumin to maintain local levels of drugs. The drug release has been controlled by the incorporation of PLGA micro and nanoparticles into gelatin constructs. The design has exhibited the potential to overcome MDR by incorporating two different types of drugs into same gelatin scaffold. Incorporated PLGA nanoparticles into gelatin scaffolds has shown higher cumulative drug release, as compared to PLGA microparticles, which shows the power of the approach such that the drug release can be controlled by using particles of different sizes during gelation. The design of polymer constructs can minimize cosmetic deformations due to surgery of breast cancer. The gelatin would degrade after a specific time and allow the healthy cells to grow and fill the cavities. In this novel design, the drugs would not cross-link with glutaraldehyde as these are loaded into particles instead of gelatin scaffolds.

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REFERENCES


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