Silk-based Biomaterials for Stabilization and Sustained Release of Neutaceuticals

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ABSTRACT SUMMARY
Silk fibroin material in a form of water-insoluble film was used as a stabilization and sustained release matrix to incorporate neutaceuticals, such as curcumin from Curcuma longa. Curcumin release kinetics, distribution, and biological activities to regulate cell growth and differentiation were examined. It was found that curcumin was stably released at a low level for more than 4 weeks from silk matrix, which promoted adipogenic differentiation of human mesenchymal stem cells (hMSCs).

INTRODUCTION
Curcumin is the yellow pigment of Curcuma longa found in turmeric spice that has antioxidant, anti-inflammatory, and anti-carcinogenic effects, thus providing potent treatments for a variety of diseases, such as cancer and aging-related diseases1. However, clinical application as well as fundamental study of curcumin encountered problems of drug solubility, stability, and bioavailability. One of the promising solutions is to develop biomaterial-based delivery systems for curcumin. Use of antioxidant-functionalized biomaterial may also interfere with cell senescence and differentiation and thus offer a valuable model system for studies of matrix-cell interactions to understand mechanisms in cell aging and tissue regeneration processes.

Silk fibroin protein from silkworm cocoons presents many physicochemical features distinguishable from other biomaterials, such as high mechanical strength, excellent biocompatibility, slow degradation, and nontoxic degradation products (amino acids)2. Silk protein can be easily manufactured into a variety of material formats, such as nano/microspheres, hydrogels, films, and porous scaffolds, via a relatively simple ambient, all-aqueous, processing3. Curcumin has been previously embedded in silk porous scaffold and its release kinetics as well as bioactivities have been determined4. The present study focus on functional incorporation of curcumin in silk hydrogel films and its impact on stem cell growth and differentiation.

EXPERIMENTAL METHOD
Curcumin powder was dissolved in ethanol and added to silk solution (20% by volume) to give a final concentration of 5 and 10 μM. Plain ethanol was added to silk at the same ratio as a control. One millilitre of mixed solution was filter-sterilized before adding to a 24 well plate. The plate was covered and incubated at 37 °C until silk was gelled. Silk gels were then dried into films in a biosafety hood overnight. One millilitre of PBS was added to each well for release study at 37 °C. Solution was replenished daily, and curcumin concentration was determined at Ex=430/Em=530 nm.

For cell culture, silk films prepared were preconditioned with growth medium containing DMEM supplemented with 10% FBS, 1% antibiotic/antimycotic, 1% non-essential amino acids, and 2 ng/mL bFGF. Cells were seeded at a density of 3000 cells/well. When cells were ~80% confluent, differentiation was induced by switching to adipogenic medium containing high glucose DMEM supplemented with 10% FBS, 1% antibiotic/antimycotic, 1% non-essential amino acids, 1 μM dexamethasone, 50 μM indomethacin, 0.5 mM isobutylxanthine, and 5 μg/mL insulin. At desired time points, cells were lysed and mRNA was isolated for reverse transcription PCR. The cDNA obtained
was then used to determine adipogenic gene expression. Oil Red O staining was used to examine the oil droplet accumulation in cells. For this, cells were fixed in 4% neutral buffered formalin overnight at 4 °C, washed with 60% isopropanol (500 µL), and stained with Oil Red O following a standard protocol.

RESULTS AND DISCUSSION
As shown in Figure 1, except for a slight burst release for the 10 µM curcumin group in the first two days, the amount of curcumin released from both 5 and 10 µM films was kept at 0.01-0.02 µM for more than 28 days (Figure 1A), representing less than 3% of total loaded drug (Figure 1B). It is likely that most curcumin bound to silk matrix, especially the hydrophobic beta-sheet structure domains in silk molecules, as reported previously².

![Figure 1](image.png)

**Figure 1.** Release kinetics of curcumin from silk hydrogel films.

As shown in Figure 2a,b, expression of all the specific adipogenic genes evaluated were significantly upregulated in cells exposed to 5 or 10 µM curcumin in silk films for 14 days as compared to the control sample of growth medium/plain silk (control) and adipogenic medium/plain silk (AM). While no significant difference in gene expression was observed for 5 and 10 µM curcumin samples, lipid accumulation was higher in cells exposed to 10 µM curcumin films throughout the 14 day experiment (Figure 2c).

![Figure 2](image.png)

**Figure 2.** Effects of curcumin concentration and duration on adipogenic gene expression and lipid accumulation in hMSCs.

CONCLUSION
Sustained release of curcumin from silk hydrogel films for at least 28 days was achieved. Although most of curcumin loaded tightly bound to silk matrix, the amount released to the medium and/or exposed to the film surface enhanced adipogenic differentiation of human mesenchymal stem cells that were cultured on the films, demonstrating that curcumin incorporated in silk hydrogel films maintained it biological functions for a long period of time.

REFERENCES

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