Expert Opinion

Silk fibroin biomaterials for controlled release drug delivery

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Introduction: Given the benefits of polymer drug delivery implants over traditional periodic systemic administration, the development of biomaterial systems with the necessary properties (biocompatibility, degradation, stabilization, controllability) is paramount. Silk fibroin represents a promising, naturally derived polymer for local, controlled, sustained drug release from fully degrading implants and the polymer can be processed into a broad array of material formats.

Areas covered: This review provides an overview of silk biomaterials for drug delivery, especially those that can function as long-term depots. Fundamentals of structure and assembly, processing options, control points and specific examples of implantable silk drug delivery systems (sponges, films) and injectable systems (microspheres, hydrogels) from the 1990s and onwards are reviewed.

Expert opinion: Owing to its unique material properties, stabilization effects and tight controllability, silk fibroin is a promising biomaterial for implantable and injectable drug delivery applications. Many promising control points have been identified, and characterization of the relationships between silk processing and/or material properties and the resulting drug loading and release kinetics will ultimately enhance the overall utility of this unique biomaterial. The ever-expanding biomaterial 'tool kit' that silk provides will eventually allow the simultaneous optimization of implant structure, material properties and drug release behavior that is needed to maximize the cost-efficiency, convenience, efficacy and safety of many new and existing therapeutics, especially those that cannot be delivered by means of traditional administration approaches.

Keywords: biomaterials, controlled release, degradable polymer, reservoirs, silk fibroin, tissue engineering

Expert Opin. Drug Deliv. (2011) 8(6):797-811

1. Introduction

Controlled sustained drug delivery from implanted polymer drug depots offers numerous advantages over traditional periodic systemic administration, including enhanced efficacy and cost-efficiency, reduction or elimination of unwanted side effects, increased patient convenience and compliance, and drug levels that are continuously maintained in a therapeutically desirable range without peaks and valleys [1]. To meet this need, many synthetic non-degradable materials or implantable pumps have been proposed, but biodegradable implants alleviate the need for surgical removal of the material after the conclusion of therapy [2]. Naturally derived biodegradable materials can offer superior biocompatibility compared with synthetic degradable materials [3]. However, naturally derived materials frequently lack the necessary control of properties and material properties needed for long-term sustained release applications [4].

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Article highlights.

- Implantable controlled release systems offer improved release kinetics, cost-efficiency, efficacy and safety compared with traditional systemic periodic administration.
- Silk fibroin from *Bombyx mori* cocoons has unique properties for implantable drug delivery, including biodegradability, biocompatibility, control, stabilizing effects on incorporated drugs, diversity of material formats and aqueous, ambient processing options.
- Silk fibroin is purified from *B. mori* cocoons by boiling in alkaline solution to remove glue-like sericin and solubilizing in hot salt followed by dialysis to remove salt.
- Degummed silk fibers have been used for a variety of applications, including tissue engineering scaffolds and fibrous medical materials, and have been directly loaded with antibiotics.
- Silk has been processed into various tissue engineering scaffolds with highly controllable morphologies and degradation behaviors, including porous three-dimensional sponges and extracellular matrix-mimicking electrospun nanofiber mats.
- Bioactivity of glucose oxidase (GOx), IGF-I and nerve growth factor (NGF) was retained when loaded in porous three-dimensional silk sponges produced using the various manufacturing techniques.
- Electrospun silk mats bulk loaded with bone morphogenetic protein 2 (BMP-2) enhanced osteogenic differentiation of human mesenchymal stem cells.
- Silk films can be used as either simple monolithic bulk-loaded slabs or coatings and have controllable properties based on drying and processing conditions.
- Controlled release of small molecules and proteins from bulk-loaded silk films has been demonstrated, as has enzyme stabilization via immobilization in silk films.
- Silk coatings have been used as nanothin film drug carriers of various model compounds as a means to improve release kinetics, mechanical strength, biocompatibility and cell–biomaterial interfaces of other biomaterial systems and as a reservoir encapsulating membrane for sustained release.
- Numerous processing techniques have been used to produce controllable silk microspheres and nanoparticles (with diameters ranging from 300 nm up to 440 μm) to deliver various drugs, including bioactive growth factors.
- Silk fibroin hydrogels with tightly controllable material properties and gelation times have been formed by sol-gel transition and have been used to sustain release of benfotiamine and buprenorphine.
- Surface modification (both via physical adsorption or chemical immobilization) can be used to attach therapeutics of interest to silk implants for degradation-dependent drug release.
- Direct adsorption has been used to load basic fibroblast growth factor (bFGF) and BMP-2 successfully into silk scaffolds.
- Chemical attachment has been used to decorate silk scaffolds with BMP-2 and fibroblast growth factor 2 (FGF-2) and was used to create stable immobilized horseradish peroxidase gradient patterns within three-dimensional silk fibroin sponges.
- Various composite materials (including multiple silk material formats integrated into single implants, combinations of silk with other polymers, and drug-releasing cells seeded on silk support matrices) show promise for sustained drug delivery.
- The broad range of material formats of silk available for implantable or injectable drug delivery combined with its unique properties and controllability make it a promising biomaterial for controlled, sustained release applications.
- Although further work is needed to characterize relationships between processing and release behavior and to correlate *in vitro* release results with *in vivo* performance, silk biomaterials are expected to provide new delivery strategies for much-needed therapeutics.

This box summarizes key points contained in the article.

Silk fibroin is a biologically derived protein polymer isolated from domestic silkworm (*Bombyx mori*) cocoons that possesses unique properties that meet all the requirements for implantable drug delivery applications (**Table 1**). Silk shows excellent biocompatibility [3,5-7] and tunable, robust mechanical properties [8]. Silk degrades to non-toxic products *in vivo* and the degradation time course of silk implants can be controlled from weeks to years via regulation of β-sheet content (crystallinity) during processing [9,10]. Whereas many polymer drug carrier systems require harsh manufacturing conditions that can degrade or denature incorporated therapeutics (shear, heat, exposure to organic solvents or extreme pH), silk fibroin can be entirely aqueously processed using mild, ambient manufacturing conditions [11,12]. Silk fibroin has also been found to exert a stabilizing effect on encapsulated enzymes and other therapeutics, even at elevated temperatures [13,14].

Silk fibroin has been processed into a variety of useful material formats including porous tissue scaffolds that structurally mimic the extracellular matrix (ECM) [15] and sustained release drug carriers [16,17]. In addition, the material properties of silk fibroin biomaterials can be tightly controlled during processing and fabrication [11]. The combined diversity of material formats available for drug delivery and tight control of the various drug carriers result in a broad range of silk-based systems available for clinical and scientific applications [18].

Although other silk-based drug delivery material formats are mentioned, emphasis in this review is on implantable or injectable silk drug delivery systems capable of functioning as long-term, sustained release depots (i.e., release duration lasting days to weeks or longer). Particular emphasis is placed on control of the silk drug delivery systems reviewed to demonstrate how the tunability of silk material properties can be used to achieve specific target release profiles.

Table 1. Summary of the advantages of silk fibroin for drug delivery.

Properties of silk fibroin
Biocompatible – FDA-approved, little immunogenicity, cytotoxicity or inflammation
Biodegradable – degradation lifetimes controllable via β-sheet content/crystallinity; degradation products are amino acids
Excellent mechanical properties
Aqueous and ambient processing or solvent processing options
Stabilizing effects on incorporated therapeutics
Facile chemical functionalization
Diverse material formats
Highly controllable material properties and release kinetics

2. Silk fibroin biomaterials for drug delivery

Bombyx mori cocoon silk consists of hydrophilic 'glue-like' sericin proteins and the hydrophobic structural protein fibroin, which consists of a heavy chain (~ 390 kDa) and a light chain (~ 25 kDa) [19,20]. Silk fibroin's heavy chain is composed of large hydrophobic blocks, much smaller hydrophilic blocks and two large hydrophilic blocks at the chain ends at the N and C termini [20,21].

Silk fibroin's primary structure is dominated by the amino acids glycine, alanine, serine, valine and tyrosine with characteristic repetitive sequences of GAGAGS, GAGAGY and GAGAGVGY [20]. These structural elements (large hydrophobic domains consisting of short side chain amino acids) permit tight packing of stacked sheets of hydrogen-bonded antiparallel chains, forming the characteristic antiparallel β-sheet secondary structure that gives silk fibroin its strength and resilience [11]. β-Sheet stacking occurs such that methyl groups and hydrogen groups of opposing sheets interact to form the inter-sheet stacking in the crystals. Strong hydrogen bonds and van der Waals forces generate a structure that is thermodynamically stable. The inter- and intra-chain hydrogen bonds form between amino acids perpendicular to the axis of the chains and the fiber [11,19].

For use in biomedical applications, silk fibroin must be purified from the glue-like sericins by degumming in boiling alkaline solution (most commonly sodium carbonate) [8,11]. Separation is required as the sericin proteins in association with fibroin have been shown to cause an inflammatory response [3]. Degummed silk fibers can be used to manufacture fiber biomaterials including yarns, sutures, rope and woven fabrics [22]. However, most drug carriers are prepared from regenerated silk fibroin, which is prepared by solubilizing the degummed silk fibroin in hot salt solution (dissolution temperature varies, typically ranging from 50 to 70°C), then dialyzing out the salt to obtain an aqueous solution (**Figure 1**). Although materials fabricated from regenerated silk fibroin can be mechanically inferior to the native silk fiber, they show superior mechanical properties compared with similar scaffolds or drug carriers prepared from other biomaterials [23,24]. In this review, degummed native silk fibers are referred to as silk fibers, whereas the silk protein in carriers prepared from regenerated silk solution is referred to as silk fibroin.

Regenerated silk fibroin solution can be processed into porous silk sponges, silk films, nano- or microscale coatings, hydrogels and nano- and microparticles (**Figure 2**). For solvent processing, regenerated silk fibroin solution can be lyophilized and then dissolved in solvent (usually 1,1,1,3,3,3-hexafluoro-2-propanol [HFIP]). Depending on the intended application and the properties of the active compound to be delivered, drug loading of silk biomaterials is typically achieved by: (i) 'bulk loading' (i.e., mixing drug and silk solutions before material fabrication); (ii) surface decoration with the drug(s) of interest (by means of either chemical coupling or adsorption); or (iii) use of composite systems.

Biodegradable drug carriers such as silk fibroin show release rates dependent on diffusion of drug through the silk, degradation of the silk polymer carrier, or a combination of both mechanisms [25]. The contribution that degradation plays in the overall release profiles depends on the diffusivity of the drug through the polymer carrier as well as the rate of degradation of the carrier. For low-molecular-mass drugs free diffusion results in rapid release, whereas higher molecular mass molecules or bound/adsorbed proteins are less able to diffuse and rely more on degradation to liberate the entrapped drug [25].

Diffusion can be controlled by means of carrier morphology (geometry, number/thickness of coating, porosity) and manipulation of polymer properties, including molecular mass and crystallinity/β-sheet content. Degradation behavior of silk is dependent on many factors, including silk processing and material properties (organic solvent versus aqueous processing, crystalline content, silk concentration and porosity [9,10]), silk material format [26,27], type and concentration of enzyme [26] and immune system response [10].

Silk in its prespun water-soluble state (referred to as silk I) is relatively unstable, and when converted to the more energetically stable, β-sheet rich silk II (via shearing, drawing, heating, spinning, exposure to solvent, etc.) the transition is considered essentially irreversible [19]. *In vivo*, glandular silk is assembled into highly ordered, β-sheet rich fibers via extraction of water, changes in pH and salt concentration and mechanical stresses during fiber spinning that induce chain alignment [28]. These processes (along with others that manipulate the factors involved in the assembly process) have been biomimetically exploited *in vitro*. For example, silk fibroin films that are cast and air-dried overnight at ambient conditions retain their silk I conformation (and water solubility) [19]. Once the films are treated with methanol, localized dehydration results in β-sheet formation by pulling off the ordered water molecules that surround fibroin's hydrophobic moieties in the solution state [20]. Stretching of the films can mimic

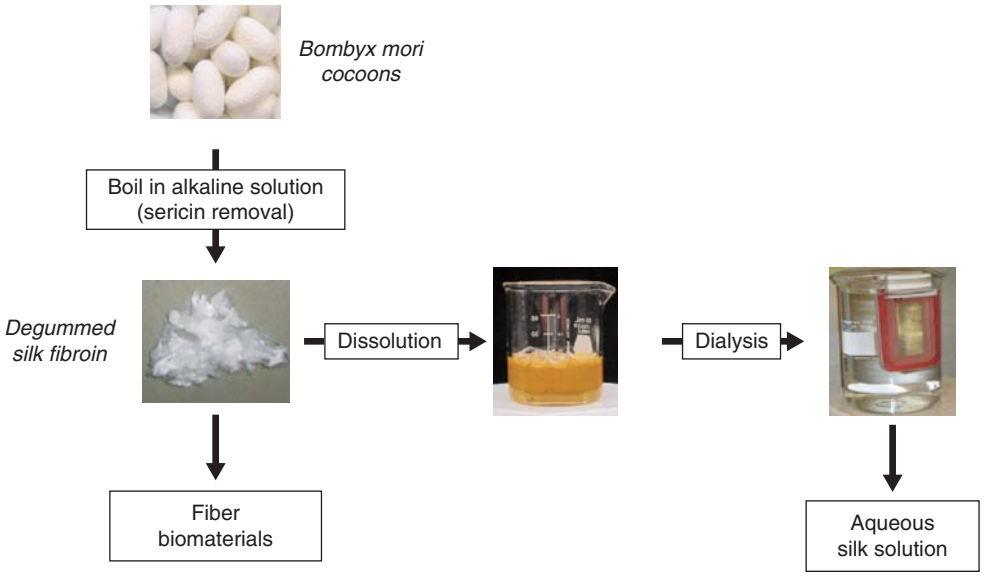

Figure 1. Silk fibroin preparation. *Bombyx mori* cocoons are purified from sericin by means of boiling in an alkaline solution. Degummed silk fibroin can be processed into fiber biomaterials including sutures, meshes, woven fabrics, yarns and ropes. To prepare aqueous silk solution, degummed silk fibroin is dissolved in salt (in this image, lithium bromide), then the salt is removed with dialysis. The aqueous silk solution can then be processed into various material formats for drug delivery.

the stresses encountered in the spinning process, forming β-sheets by inducing chain alignment. These manipulations affect silk secondary structure and material properties, which in turn affect drug release behavior.

2.1 Degummed silk fibers and fabrics

Degummed silk fibers have been processed into various material formats (including woven fabric, yarns, ropes and knitted scaffolds) for a range of biomedical applications, including soft-tissue reinforcement meshes [17], sutures [8], and tissue-engineered ligaments [29], tendons [30] and blood vessels [31]. Notably, Altman *et al.* confirmed the utility of silk fibers for ligament tissue engineering by matching the complex and demanding mechanical requirements of a native human anterior cruciate ligament (ACL) and demonstrating support of cell attachment, expansion and differentiation [32].

Drug-eluting silk fibers could be used to enhance the therapeutic efficacy of biomedical fabrics, yarns and knitted contructs. Choi *et al.* 'dyed' silk fibers with two antibiotics (doxycycline and ciprofloxacin) using a range of conditions, including varied dyeing temperature, duration and dyebath pH, and treatment of silk with NaOH for varied times to induce chemical and conformational changes. Varied processing and differences in the antibiotic properties affected the total drug loading and release behavior. Fibers loaded with doxycycline and ciprofloxacin were able to inhibit local growth of *Staphylococcus epidermis* (measured using a zone of inhibition assay compared with a standard antibiotic-impregnated Sensi-Disc) for at least 48 and 24 h, respectively [33].

2.2 Tissue engineering scaffolds

2.2.1 Porous three-dimensional sponges

Three-dimensional porous silk fibroin sponge scaffolds can be prepared from regenerated silk solution using either aqueous or organic solvent (HFIP) processing. The necessary interconnected pores in the sponges can be generated using salt leaching, gas foaming or freeze-drying [34,35]. The morphological and structural features of the scaffolds produced by salt leaching depend on several variables, including silk fibroin concentration, solid salt particle loading, salt particle size, and the use of aqueous- or HFIP-derived processes [36]. Degradation behavior of silk sponges is also affected by processing (pore size, aqueous- or HFIP-derived processes, silk concentration) [10,37,38]. The pore size of sponges made from lyophilized hydrogels is dependent on silk fibroin concentration, gelation temperature and Ca^{2+} concentration [39].

Incorporation of drug delivery into porous tissue engineering scaffolds can provide high local concentrations of spatiotemporally controlled signaling growth factors. Although silk sponges have predominantly been studied as tissue engineering scaffolds, the diversity of processing options and tight control of the resulting structural and mechanical properties suggest silk sponges might show a similar degree of control as a drug carrier.

Aspirin-loaded silk fibroin foams prepared by freeze-drying an aqueous solution of regenerated silk and aspirin showed burst release followed by constant release lasting ~ 2.5 days. Average pore size and morphology of the silk foam were dependent on freezing temperature, pH and methanol treatment [40]. Lyophilized silk fibroin hydrogel matrices

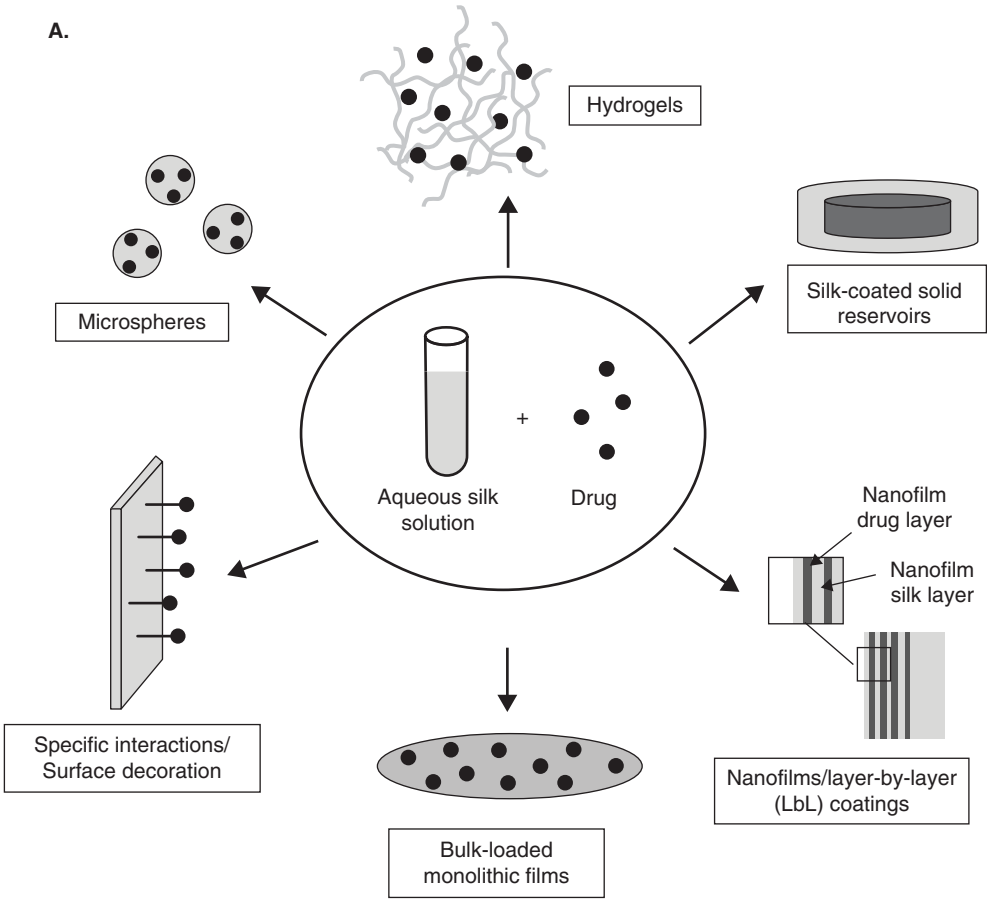

Figure 2. Silk material formats for drug delivery. A. Schematic representations of silk drug carriers. **B.** Examples of silk drug delivery biomaterials. **a.** Degummed silk fibers loaded with the antibiotic rifampicin inhibit growth of a bacterial lawn. **b.** Porous sponges (empty/unloaded on the left; loaded with Nile red on the right) and histology sections of silk sponges implanted in a rat brain (Crystal violet stain). **c.** Bulk-loaded film containing indigo carmine dye. **d.** Sonication-induced gel containing phycoerythrin. **e.** Microspheres coated with fluorescein-coupled silk and silk film loaded with Nile red via adsorption. **f.** Silk-encapsulated reservoir and SEM images of a silk dip-coating layer on a silk film substrate. **g.** Red fluorescent bead-loaded silk microspheres and SEM of silk microspheres.
SEM: Scanning electron microscopy.

loaded with monoclonal antibodies showed sustained release for up to 38 days. Antibody release was governed primarily by hydrophobic/hydrophilic silk–antibody interactions and secondarily altered by hydration resistance related to β-sheet (crystalline) density of the matrix [41].

Highly porous three-dimensional silk scaffolds loaded with insulin-like growth factor I (IGF-I) were fabricated using freeze-drying and porogen leaching, achieving sustained release of bioactive growth factor from a substrate suitable for tissue growth. Methanol treatment after freeze-drying increased β-sheet content in the scaffolds, reduced initial burst, achieved higher total cumulative IGF-I release and increased release duration. Sustained growth factor release from scaffolds enhanced chondrogenic differentiation of human mesenchymal stem cells (hMSCs) cultured on IGF-loaded silk scaffolds, compared with no chondrogenic response for hMSCs cultured on unloaded control silk scaffolds [42]. Nerve growth factor (NGF)-loaded porous tube-shaped scaffolds for nerve guides were prepared by filling molds with an aqueous solution of NGF and silk, freezing-drying (freezing at -20 or -196°C) and methanol treating to induce β-sheet formation and water insolubility of the silk matrices. Methanol treatment did not alter NGF bioactivity and increased the proportion of bound NGF. NGF release was prolonged over 3 weeks and supported PC12 cell differentiation with neurite outgrowth. Freezing temperature (-20 or -196°C) influenced carrier porosity, morphology, release rate and absolute cumulative NGF release [43].

2.2.2 *Electrospun nanofiber mats*

Electrospinning is a process that produces nanoscale fibers (diameters from 50 to 500 nm) with microscale-interconnected

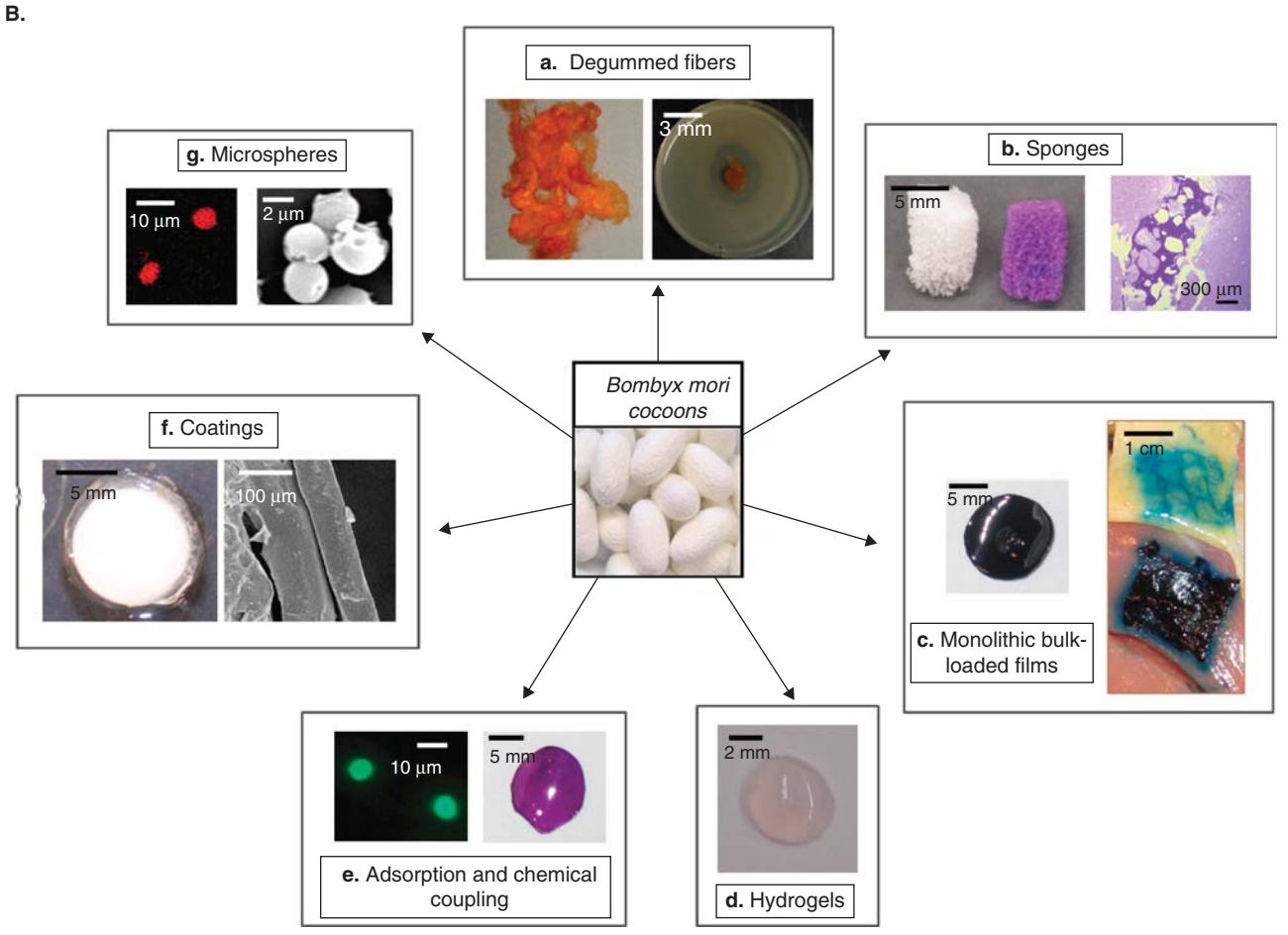

Figure 2. Silk material formats for drug delivery (continued). A. Schematic representations of silk drug carriers. **B.** Examples of silk drug delivery biomaterials. **a.** Degummed silk fibers loaded with the antibiotic rifampicin inhibit growth of a bacterial lawn. **b.** Porous sponges (empty/unloaded on the left; loaded with Nile red on the right) and histology sections of silk sponges implanted in a rat brain (Crystal violet stain). **c.** Bulk-loaded film containing indigo carmine dye. **d.** Sonication-induced gel containing phycoerythrin. **e.** Microspheres coated with fluorescein-coupled silk and silk film loaded with Nile red via adsorption. **f.** Silk-encapsulated reservoir and SEM images of a silk dip-coating layer on a silk film substrate. **g.** Red fluorescent bead-loaded silk microspheres and SEM of silk microspheres.
SEM: Scanning electron microscopy.

pores [44]. Nanofiber mat scaffold properties (geometry, fiber diameter, orientation and porosity) can be controlled by manipulation of the solution properties and operating parameters [44]. This controllability and the structural similarity to native ECM make electrospun silk fibroin biomaterials excellent substrates for tissue engineering [45]. Electrospun silk nanofiber mats were bulk-loaded with bone morphogenetic protein 2 (BMP-2) and/or hydroxyapatite nanoparticles (nHAP) by mixing the growth factor and nanoparticles into the aqueous silk solution before electrospinning. The mild aqueous processing resulted in retention of the activity of the encapsulated BMP-2, as evidenced by increased osteogenesis of hMSCs cultured for 31 days on BMP-2-loaded scaffolds compared with empty control scaffolds [46].

2.3 Silk fibroin films: monolithic slabs and coatings

2.3.1 Bulk-loaded monolithic silk films

Bulk loading of silk fibroin films represents a straightforward method for preparing monolithic drug delivery implants: regenerated silk solution is mixed with the drug solution of interest, and films of the desired thickness and surface area are cast, dried, and then treated to produce the desired material and release properties. Silk fibroin film-processing options include controlled slow drying [47], water annealing [48], stretching, compressing [49] and solvent immersion (including methanol [28], ethanol [50], glutaraldehyde [51], 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide [EDC]) [52].

Permeability of silk fibroin films to small molecule pharmaceuticals (5-fluorouracil, vitamin C, resorcinol, sodium

phenolsulfonate and benzyltrimethylammonium chloride) is dependent on release buffer pH and drug properties [53]. Hofmann *et al.* prepared monolithic bulk-loaded films from aqueous silk solution containing dextrans of different molecular masses (4, 10, 20 and 40 kDa) and horseradish peroxidase (HRP) and lysozyme (Lys) as model proteins. Release from the films was sustained for ~ 4 weeks and release behavior was related to film crystallinity and drug properties (including molecular mass and adsorption to the silk) [28]. The release of fluorescein isothiocyanate (FITC)-labeled dextrans of varying sizes (4, 10, 20 and 40 kDa) from methanol-treated and untreated silk fibroin films has also been reported. For a mechanism-based semiempirical model derived from Fickian diffusion, the estimated diffusion coefficient was smaller for the methanol-treated films than for the untreated films and decreased linearly with increasing analyte molecular mass [54]. Blended polyurethane-silk fibroin films loaded with heparin showed sustained release over 24 h and high controllability (release rate and total cumulative heparin release could be controlled by means of total heparin load in the film, composition ratio of silk fibroin to polyurethane and film thickness) [55].

The effects of various post-drying treatments (water annealing for 6 h, methanol treating for 8 min, stretching) on stabilization and release of HRP-loaded silk fibroin films and silk films prepared with 30% glycerol were examined. HRP release was linearly related to silk degradation, which can be controlled through manipulation of the silk film structure (in this case, β-sheet content related to type and duration of post-drying treatment). Along with material properties and degradation behavior, processing conditions also determined the ratio of untrapped (readily released by diffusion) and trapped protein (bound and inactive until released by proteolytic degradation). The authors also reported that, owing to the unique periodic hydrophobic-hydrophilic domains of the silk structure, HRP in films retained > 90% of the initial activity after 2 months at 37°C. This study demonstrated that silk films were capable of both retention of enzyme activity and controlled release kinetics [14].

It is worth noting that numerous studies have demonstrated that silk films can be used as a stabilizing immobilization matrix for proteins and enzymes, reducing their sensitivity to pH, temperature and proteinase-induced degradation. Proteins stabilized in silk films include glucose oxidase [13], immunoglobulin G-binding *Staphylococcal* protein A [56], uricase [57], tyrosinase [51], lipase [13], β-glucosidase [50], HRP [12,13,58], myoglobin [58], hemoglobin [12,58], catalase [58] and green fluorescent protein (GFP) [59]. These protein immobilization studies demonstrate that the activity of labile active compounds can be maintained during fabrication of silk carriers and retained during storage in silk carriers, essential criteria to be met by polymers intended for sustained drug release applications.

2.3.2 Silk coatings

The addition of silk fibroin film coatings offers many potential advantages, including controlled release drug delivery [60,61], improved release kinetics (increased diffusion barrier to drug release, decreased carrier degradation, enhanced control, and so on [60,62,63]), added mechanical strength [54] and improved cell–biomaterial interfaces [61,63-66].

Wang *et al.* developed a simple, entirely aqueous layer-by-layer (LbL) assembly technique for applying nanoscale silk fibroin film coatings to solid substrates. This stepwise deposition process resulted in robust, stable material coatings that were controllable. Film thickness increased linearly with the number of silk fibroin layers deposited, and control of individual layer thickness (ranging from a few to tens of nanometers) could be achieved via manipulation of the concentrations of silk and salt and the rinsing method [67]. Later studies validated the use of silk nanofilm coatings as a delivery vehicle for various bioactive drugs, including small molecules and proteins. Wang *et al.* prepared silk nanofilm coatings loaded with model drugs Rhodamine B, Even Blue and Azoalbumin and found that control of β-sheet content and the multilayer structure of the silk coatings could be used to regulate the release kinetics of the incorporated compounds [60]. Silk nanofilm coatings loaded with heparin, paclitaxel, and clopidogrel were validated as stent coating materials both *in vitro* and *in vivo* [61].

Wang *et al.* demonstrated that ultrathin silk fibroin coatings applied to poly(lactide-co-glycolide) (PLGA) and alginate microspheres significantly retarded release of model drugs (HRP and bovine serum albumin [BSA]), particularly when the silk coatings were methanol treated. Along with increasing the diffusion barrier, silk coatings provided mechanically stable shells and delayed degradation. Further, the process could be carried out aqueously, did not alter protein loading or microsphere morphology, and was characterized by controllable coating thickness and crystalline content [62]. Silk fibroin coatings on liposomes have also been shown to improve release kinetics, cell targeting and liposome uptake [63,68]. Silk coating on quantum dots reduced particle aggregation and improved their water solubility, clearance and biocompatibility [65,66].

Silk coatings can also be applied to solid drug dosage formats, including pills for oral delivery [52] and dry powder reservoirs for long-term release [69]. Bayraktar *et al.* examined tablets of theophylline dip-coated with thin silk fibroin films containing different amounts of polyethylene glycol (PEG) and silk films crosslinked with EDC. Silk films prepared with 17% PEG or crosslinked with EDC produced zero-order release of theophylline over 5 and 7 h, respectively. Varied PEG concentration and use of multiple EDC-crosslinked coatings allowed tuning of drug release kinetics [52]. Press-fit tablets prepared from blends of silk powder and theophylline for oral delivery showed decreasing release rate as the fibroin content in the tablets increased [70]. Press-fit pills of the small molecule anticonvulsant adenosine were coated with thicker silk fibroin films (~ 0.1 to ~ 0.8 mm, depending on the silk concentration) by dipping, drying, then methanol treating. Release behavior from these silk-encapsulated reservoirs was

related to coating thickness (controllable through silk coating solution concentration and number of coatings applied), protease degradation rate and crystallinity/β-sheet content of the silk film coating [69]. In the case of encapsulated reservoirs coated with 8 layers of 8% (w/v) silk, zero-order release was sustained for 14 days. Silk fibroin-encapsulated solid powder reservoirs were also prepared to co-release both adenosine and ethylenediamine tetraacetic acid (EDTA). Release of EDTA from the reservoirs inhibited proteolysis of the silk coating and delayed adenosine release in protease buffer, suggesting that drug delivery might also be controllable through regulation of local proteolytic activity and degradation rate [71].

2.4 Microspheres and nanoparticles

Silk fibroin microspheres have been prepared using a variety of methods, including use of lipid template [72], spray-drying [73-75], ethanol and freezing-induced self-assembly [76-78], salting-out [79], water-in-oil emulsification [80], laminar jet break-up of an aqueous silk solution [81] and casting and dissolution of PVA-silk blend films [82]. Though silk fibroin nanoparticles have been fabricated successfully [16,64,83], nanoparticles are less well suited to depot applications as their small size and high surface area-to-volume ratio leads to rapid drug release.

Wang *et al.* developed a method to prepare silk fibroin microspheres < 2 μm in diameter by adding aqueous silk solution to phospholipid, freeze-drying and treating with methanol or sodium chloride to remove the lipid vesicle template and induce β-sheet formation in the silk. Release duration of HRP from these microspheres varied from 10 to 15 days depending on duration of sodium chloride treatment, demonstrating that release kinetics can be controlled via processing [72].

Silk microspheres produced by ethanol precipitation showed predictable sizes and size distributions (ranging from 0.2 to 1.5 μm) that could be controlled through manipulation of preparation conditions, including amount of ethanol additive, freezing temperature and concentration of silk fibroin solution [76]. Bessa *et al.* took advantage of the mild processing conditions associated with this approach to encapsulate growth factors. They demonstrated successful loading and sustained release of therapeutically relevant amounts of BMP-2, BMP-9 and BMP-14 over 14 days [77]. In a later study, the authors confirmed the bioactivity of the BMP-2 released from these silk microspheres *in vitro* and *in vivo* [78].

Salting-out represents another simple aqueous preparation method for silk microparticles. Lammel *et al.* used potassium phosphate salting-out to prepare smaller silk fibroin particles (~ 0.5 – 2 μm). Secondary structure and particle size could be controlled by means of pH and protein concentration, respectively. Particles were loaded post-fabrication with small molecule model drugs (Alcian blue, Rhodamine B and Crystal violet) by simple adsorption [79].

Wenk *et al.* fabricated drug-loaded silk fibroin spheres using the laminar jet break-up of an aqueous silk solution (induced by nozzle vibration at controlled frequency and amplitude) followed by methanol treatment or water vapor annealing to increase β-sheet content. The resulting spheres were 101 – 440 μm in diameter with sizes dependent on nozzle diameter and processing conditions. Salicyclic acid, propanolol hydrochloride and IGF-I-loaded microspheres showed release durations of 1 day, 20 days and > 7 weeks, respectively. This process was characterized by high encapsulation efficiency (close to 100% before methanol treatment), retention of bioactivity and controllable release kinetics that were tunable via silk concentration [81].

Recently, Wang *et al.* developed an easy, efficient, aqueous-based method for micro- and nanosphere preparation based on the phase separation of blended silk fibroin and polyvinyl alcohol (PVA) [82]. Size (ranging from 300 nm up to 20 μm) and polydispersity could be controlled by manipulation of the silk-to-PVA ratio or the application of ultrasonication. In addition, they demonstrated loading and release of model drugs spanning a range of sizes, hydrophobicities and charges (BSA, dextran and Rhodamine B) from their silk particles, demonstrating their broad utility as drug delivery vehicles [82].

2.5 Hydrogels

β-Sheet rich, physically crosslinked hydrogels have been formed from silk fibroin solution by sol-gel transition [84]. As gelation occurs owing to the formation of inter- and intramolecular interactions among the fibroin protein chains (including hydrophobic interactions and hydrogen bonds), manipulations that increase the ability of the fibroin chains to interact (input of energy into the solution, reducing repulsion among the chains or dehydration to remove the water molecules that stabilize the hydrophobic moieties in solution) lead to increased physical crosslinking and shorter gelation times [39]. Gelation is therefore affected by temperature, silk fibroin concentration and pH [36,39], and can be accelerated by additives [36], sonication [84] or vortexing [85]. Mechanical properties [39], porosity [39,84] drug release behavior [86] and gelation time [39] can be tightly controlled through manipulation of the processing conditions. In addition, sonication and vortex-induced silk hydrogels both have a useful range of timeframes for cell, drug or microsphere encapsulation before final gel-setting [84,85]. Gelation conditions can be selected to ensure silk solution remains in a liquid state long enough to mix in other components and be injected, then completes gelling *in vivo* at the injection site.

Hanawa *et al.* prepared silk fibroin hydrogels loaded with the vitamin B_1 derivative benfotiamine (BTMP) and studied release behavior as a function of silk fibroin and glycerol content and the presence of BTMP-solubilizing β-cyclodextrin. BTMP release from the silk hydrogels was found to decrease with increasing silk fibroin concentration, firmness and glycerol concentration and the addition of β-cyclodextrin increased BTMP release and silk hydrogel firmness [86].

Fang *et al.* investigated rapid release of buprenorphine (a morphine-like analgesic) from silk fibroin hydrogels prepared from varied silk concentration and molecular mass silk proteins and found that material properties and drug release rate could be controlled via manipulation of the concentration of silk and by blending of silks of different molecular masses. Zero-order release was observed for the 4 h duration studied [87].

Hydrogels prepared from a genetically modified version of recombinant silk containing periodic incorporation of elastin-like blocks (for increased solubility and gelation rate and decreased total crystallinity and bioresorption rate) termed silk-elastinlike polymer (SELP) hydrogels have been applied to a wide range of drug delivery applications, including delivery of plasmid DNA [88], adenovirus [88-90], fluorescently labeled probes [91], cytochrome *c*, vitamin B_{12}, theophylline [92] and recombinant protein mitotoxin [91]. For reviews of synthesis, characterization and drug delivery from silk-elastinlike polymers, see [93,94].

3. Surface modification

Surface modification is frequently used in silk tissue engineering scaffolds to alter cell attachment and proliferation, especially attachment of the Arg-Gly-Asp (RGD) peptide to silk fibroin biomaterial surfaces to increase cell attachment (including films [15], fibers [95] and sponges [96]). Surface modification (including physical adsorption or chemical immobilization) can also be used to attach therapeutics of interest to silk implants; because free diffusion of adsorbed or chemically coupled drugs from silk carriers is limited [97,98], drug release from these silk implants is more dependent on silk degradation rate.

3.1 Adsorption

Owing to the hydrophobicity of silk surfaces, proteins can be attracted or repelled depending on the pI and hydrophobicity of the protein and pH of the solution. Attraction between silk and other compounds can be used to decorate silk biomaterials by simple absorption.

Lipase immobilized by means of adsorption on silk fibers showed enhanced pH and temperature stability and increased enzymatic activity [99]. Three-dimensional porous silk fibroin sponges fabricated using either the aqueous or the HFIP salt-leeching technique were loaded with basic fibroblast growth factor (bFGF) by dropping bFGF solution onto the sponges and allowing adsorption to occur overnight at 4°C. The affinity between the bFGF and the silk substrate resulted in incomplete drug release *in vitro* (~ 30% over 3 days), but exposure to proteinase induced silk matrix degradation, increasing total cumulative release and release duration (~ 85 – 90% over 8 days). When immersed in proteinase, the HFIP-derived scaffolds degraded (and thus released bFGF) more slowly than the aqueous-derived scaffolds. *In vivo*, injected bFGF disappeared within 24 h of administration whereas implanted bFGF-loaded silk fibroin sponges sustained release over 14 days [97]. Karageorgiou *et al.* adsorbed BMP-2 onto silk films by covering the silk films with a BMP-2 solution for 2 h at room temperature followed by rinsing and drying. Adsorbed BMP-2 on silk films caused an increase in hMSC osteogenesis compared with controls (unmodified silk films), though less of an increase in hMSC osteogenesis than covalently coupled BMP-2 on silk films [98].

3.2 Chemical immobilization

Silk fibroin can be functionalized using the amino acid side chain chemistry, particularly carbodiimide chemistry, which uses amine or carboxyl groups on silk for modification. Karageorgiou *et al.* used carbodiimide chemistry to immobilize BMP-2 directly onto silk fibroin films. Covalently coupled BMP-2 remained bioactive and was retained on the film surface longer than adsorbed BMP-2 (50% remaining bound after 4 weeks in culture media, compared with only 10%). Increased osteogenesis was observed for hMSCs seeded on the surface of BMP-2-decorated films compared with hMSCs exposed to similar amounts of soluble BMP-2 supplementation in the media, which the authors attribute to enhanced stability and higher protein concentrations in the local microenvironment [98]. Wenk *et al.* used diazonium coupling to decorate silk fibroin films with a sulfonated moiety to bind fibroblast growth factor 2 (FGF-2) and found that silk films decorated with sulfonic acid showed high, controlled binding of bioactive FGF-2 [100]. NeutrAvidin was coupled to silk fibroin in solution and the silk retained its self-assembly features post-reaction. NeutrAvidin was also coupled to silk microspheres and then functionalized further with biotinylated anti-CD3 antibody, resulting in specific binding of the functionalized microspheres to CD3-positive T-lymphocytic cells [101].

Vepari and Kaplan generated stable immobilized HRP gradients within three-dimensional silk fibroin sponges by activating the HRP with carbodiimide then distributing the activated HRP using either diffusion or convection coupled with diffusion. Slope and activity of the immobilized HRP gradients were controlled by varying the volume and starting concentration of activated HRP solution. Covalent immobilization of HRP increased enzyme stability versus time and temperature compared with adsorbed HRP. This simple, mild process could be extended to gradient immobilization of a variety of proteins or small molecules [102].

For more information on conjugation of drugs to silk fibroin biomaterials and chemical modification of silk fibroin for biomedical applications, see [103].

4. Composite materials

Biomaterials fabricated by integrating multiple material formats (composite materials) offer numerous advantages,

including enhanced control of release kinetics and the possibility to combine both precise control of temporal and spatial biological signals and physical/mechanical cues in a single construct. For an excellent review of silk-based composite biomaterials, see [104]. Madduri *et al.* prepared silk fibroin films to release glial cell line-derived neurotrophic factor (GDNF) and NGF and incorporated them into nerve guidance conduits. The conduits showed sustained release of GDNF and NGF *in vitro* over 4 weeks, and when cultured with various neuronal cells from chicken embryos induced an augmented length and rate of axonal outgrowth parallel to the aligned nanofibers [105]. To achieve controlled spatial distribution of multiple growth factors in a tissue engineering scaffold, Wang *et al.* prepared silk fibroin microspheres loaded with BMP-2 and IGF-I using the lipid-template technique and incorporated them into aqueous-derived silk porous scaffolds using a gradient process. Both growth factors formed deep and linear concentration gradients in the scaffolds and were shown to induce and control hMSC differentiation [106].

To increase release duration of the small molecule neuroprotectant adenosine from silk implants, multiple silk fibroin biomaterials were integrated into a single construct [107]. Implants were fabricated by preparing adenosine-releasing silk microspheres using the lipid template technique [72], embedding them in aqueous-derived silk porous rods, then coating with adenosine-loaded silk films to increase the diffusion barrier, mechanically reinforce the implants and increase total drug loading. Implants prepared using varied microsphere concentrations in the scaffold and varied adenosine concentrations in the silk film dipping solutions resulted in different release rates *in vitro*. When these rods were implanted in the hippocampi of kindled epileptic rats, a dose-dependent delay in seizure acquisition was observed [107]. Later studies demonstrated that silk implants designed to release 1000 ng adenosine a day completely protected kindled rats from seizures over a 10-day period, which agreed with the release duration observed in a release study conducted in 37°C phosphate buffered saline [108]. These studies demonstrate that sustained, local adenosine delivery from silk-based implants represents a safe and efficient strategy to suppress seizures and possibly delay epileptogenesis.

Numerous composite biomaterial scaffolds based on combining silk biomaterials with other polymer drug delivery systems have also been developed, including PLGA [109], gelatin [110], polyacrylamide [111] and alginate [112,113]. Wenk *et al.* prepared silk fibroin sponges carrying embedded PLGA microspheres to release IGF-I and found that embedment in the silk scaffolds led to slower release rates (~ 80 – 100% total IGF-I release after 50 days from free microspheres, compared with ~ 20 – 40% from composite scaffolds) [109]. Mandal and Kundu prepared porous silk scaffolds (using the freeze-drying method and silk from Indian non-mulberry tropical tasar silkworm, *Antheraea mylitta*) embedded with calcium alginate microspheres and calcium alginate/silk fibroin-blended microspheres for controllable, dual protein release. Release behavior of the model proteins tested (BSA and FITC-inulin) was dependent on composition of the microspheres and silk content (in weight per cent) of the scaffold, suggesting release kinetics can be tightly controlled. Embedding microspheres in silk sponges provided a mechanically stable shell and increased the diffusion barrier to the encapsulated protein drugs [113]. Controlled drug release from multilayer films based on a blend of silk fibroin and self-degradable gelatin has been studied. Release of model compounds (Trypan blue, FITC-inulin and FITC-BSA) was dependent on the ratio of silk fibroin to gelatin and the build-up of layers. Modeling of release behavior suggested a combined mechanism of Fick's diffusion and polymer degradation [110].

Owing to the ability of silk to support cell growth, silk biomaterials are also useful for cell-mediated drug delivery. Modified bone marrow stromal cells (BMSCs) transduced with an adenovirus overexpressing BMP-2 were seeded on premineralized aqueous-derived silk scaffolds to repair mandibular defects in a rat model. The premineralized scaffold alone alone did not result in repair, whereas the premineralized scaffolds seeded with BMP-2 expressing bMSCs increased new bone formation and local bone mineral density [114]. Silk fibroin films were shown to be suitable substrates for adenosine-releasing adenosine kinase-deficient (Adk-/-) embryonic stem cells. Differentiation of Adk-/- embryonic stem cell (ESC)-derived glial precursor cells was efficient on silk films and the amounts of adenosine released by the cell cultures on silk substrates (420 ng/ml) were considered to be of therapeutic relevance [115].

5. Conclusions

Silk has attractive properties for controlled, sustained release. Silk can be aqueously processed into a diverse range of material formats for implantable or injectable sustained release drug depots, possesses remarkable mechanical properties, biodegrades over controllable timeframes and preserves stability during encapsulation and storage. Silk biomaterials, with their excellent material properties, unique and versatile processing options, biocompatibility and highly tunable material properties, are poised to affect significantly not only drug delivery, but also many other biomedical applications, including tissue engineering, implant coating, imaging and diagnostics.

6. Expert opinion

Implantable polymer systems capable of achieving controlled, sustained release of drugs locally have the potential not only to improve existing therapies, but also to deliver drugs that until now have been challenging or impossible to administer owing to rapid clearance, poor stability or failure to reach the site of action via systemic delivery. Despite these advantages, polymers that meet the necessary requirements for successful, broadly applicable controlled

release devices have remained elusive. PLGA systems have received considerable attention owing to their ability to degrade *in vivo* and their customizable degradation rates, but PLGA systems induce local inflammation and suffer from poor drug stabilization (owing to decreased local pH and use of organic solvents during encapsulation/material fabrication). Natural degradable polymers often offer superior biocompatibility to synthetic degradable polymers, but cannot achieve the controllable release profiles and sustained release duration of their PLGA counterparts.

So far, research in the field of silk-based drug delivery has demonstrated the exceptional potential of this unique biomaterial to overcome the limitations that have prevented other polymer controlled release implants from achieving widespread medical utility. The diversity of drugs and silk material formats reported in the literature suggests that these properties can be exploited to meet a multitude of urgent clinical needs. Further, numerous material processing control points, such as silk solution concentration and β-sheet content, among others, have been identified for each of the silk material formats described in this review, suggesting that release behavior can be highly tunable. Although many promising control points have been identified, further work is needed to characterize the relationships between silk processing and/or material properties and the resulting drug loading and release kinetics. The ultimate goal of the field is to characterize these relationships and translate this understanding into highly controlled systems. Emphasis will probably be placed on implants that achieve sustained zero-order or pulsed-release kinetics, but other release profiles will also have clinical significance. The pursuit of thorough, rigorous characterization of the fundamental driving forces behind drug release from silk (along with continued research focused on specific applications) will ultimately enhance the overall utility of this unique biomaterial for drug delivery and for building controlled release into other biomedical materials (implant coatings, tissue engineering scaffolds, drug storage matrices, optical devices, and so on).

As with any implantable drug delivery system, there is also a critical need to relate *in vitro* release behavior with *in vivo* performance; because silk degrades via proteolysis and local enzymatic activity may vary from implantation site to implantation site, it will be especially important to investigate the effects of *in vivo* degradation and strategies to control local degradation (as the authors have recently described [71]). In the case of adenosine-releasing silk rods [107,108], the authors expect that the unique properties of the system and the tight correlation of the *in vitro* release profiles with *in vivo* results in animal studies will result in rapid translation into therapies for pharmacoresistant epilepsy and other adenosine-related disorders. They are also confident that this success will not be limited to adenosine augmentation therapies. The ever-expanding biomaterial 'tool kit' silk provides will eventually allow the simultaneous optimization of implant structure, material properties and drug release behavior that is needed to maximize the cost-efficiency, convenience, efficacy and safety of many new and existing therapeutics, especially those that cannot be delivered by traditional administration approaches.

Declaration of interest

DL Kaplan declares a commercial conflict of interest with Ekteino, Inc. EM Pritchard declares no conflict of interest.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Langer R. Invited review: polymeric delivery systems for controlled drug release. Chem Eng Commun 1980;6:1-48
2. Danckwerts M, Fassih A. Implantable controlled release drug delivery systems: a review. Drug Dev Ind Pharm 1991;17:1465-502
3. Panilaitis B, Altman G, Chen J, et al. Macrophage responses to silk. Biomaterials 2003;24:3079-85
4. Puppi D, Chiellini F, Piras AM, Chiellini E. Polymeric materials for bone and cartilage repair. Prog Polym Sci 2010;35:403-40
5. Leal-Egana A, Scheibel T. Silk-based materials for biomedical applications. Biotechnol Appl Biochem 2010;55:155-67
6. Tang X, Ding F, Yang Y, et al. Evaluation of in vitro biocompatibility of silk fibroin-based biomaterials with primarily cultured hippocampal neurons. J Biomed Mater Res A 2009;91A:166-74
7. Meinel L, Hofmann S, Karageorgiou V, et al. The inflammatory responses of silk films in vitro and in vivo. Biomaterials 2005;26:147-55
8. Altman GH, Diaz F, Jakuba C, et al. Silk-based biomaterials. Biomaterials 2003;24:401-16
9. Horan RL, Antle K, Collette AL, et al. In vitro degradation of silk fibroin. Biomaterials 2005;26:3385-93
10. Wang Y, Rudym DD, Walsh A, et al. In vivo degradation of three-dimensional silk fibroin scaffolds. Biomaterials 2008;29:3415-28
11. Vepari C, Kaplan DL. Silk as a biomaterial. Prog Polym Sci 2007;32:991-1007

• **Review of silk material formats and processing options for tissue engineering and drug delivery applications.**

12. Lawrence BD, Cronin-Golomb M, Georgakoudi I, et al. Bioactive silk protein biomaterial systems for optical devices. Biomacromolecules 2008;9:1214-20
13. Lu S, Wang X, Lu Q, et al. Stabilization of enzymes in silk films. Biomacromolecules 2009;10:1032-42
14. Lu Q, Wang X, Hu X, et al. Stabilization and release of enzymes from silk films. Macromol Biosci 2010;10:359-68

• **Demonstrates controllable protein release from silk films and stabilizing effects of incorporation in silk films.**

15. Sofia S, McCarthy MB, Gronowicz G, Kaplan DL. Functionalized silk-based biomaterials for bone formation. J Biomed Mater Res 2001;54:139-48
16. Numata K, Kaplan DL. Silk-based delivery systems of bioactive molecules. Adv Drug Deliv Rev 2010;62:1497-508

•• **An excellent review of synthesis of silkworm and spider silk-based drug carriers.**

17. Wenk E, Merkle HP, Meinel L. Silk fibroin as a vehicle for drug delivery applications. J Control Release 2011;150(2):128-41

•• **An excellent review of relevant silk fibroin properties and fabrication processes that affect drug release from silk fibroin drug carriers, including specific examples of silk-controlled release drug delivery systems.**

18. Omenetto FG, Kaplan DL. New opportunities for an ancient material. Science 2010;329:528-31
19. Kaplan DL, Mello CM, Arcidiacono S, et al. Silk. In: McGrath K, Kaplan D, editors, Protein-based materials. Birkhäuser, Boston; 1997
20. Matsumoto A, Chen J, Collette AL, et al. Mechanisms of silk fibroin sol-gel transitions. J Phys Chem B 2006;110:21630-8
21. Kim U-J, Park J, Li C, et al. Structure and properties of silk hydrogels. Biomacromolecules 2004;5:786-92
22. Horan RL, Collette AL, Lee C, et al. Yarn design for functional tissue engineering. J Biomech 2006;39:2232-40
23. Kim UJ, Park J, Kim HJ, et al. Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin. Biomaterials 2005;26:2775-85
24. Kluge JA, Rosiello NC, Leisk GG, et al. The consolidation behavior of silk hydrogels. J Mech Behav Biomed Mater 2010;3:278-89
25. Luo Y, Kirker KR, Prestwich GD. Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. J Control Release 2000;69:169-84
26. Arai T, Freddi G, Innocenti R, Tsukada M. Biodegradation of Bombyx mori silk fibroin fibers and films. J Appl Polym Sci 2004;91:2383-90
27. Yang Y, Zhao Y, Gu Y, et al. Degradation behaviors of nerve guidance conduits made up of silk fibroin in vitro and in vivo. Polym Degrad Stabil 2009;94:2213-20
28. Hofmann S, Wong Po Foo CT, Rossetti F, et al. Silk fibroin as an organic polymer for controlled drug delivery. J Control Release 2006;111:219-27

• **One of the first studies of controllable silk drug delivery systems and the effects of material properties on release behavior.**

29. Liu H, Fan H, Wang Y, et al. The interaction between a combined knitted silk scaffold and microporous silk sponge with human mesenchymal stem cells for ligament tissue engineering. Biomaterials 2008;29:662-74
30. Chen JL, Yin Z, Shen WL, et al. Efficacy of hESC-MSCs in knitted silk-collagen scaffold for tendon tissue engineering and their roles. Biomaterials 2010;31:9438-51
31. Enomoto S, Sumi M, Kajimoto K, et al. Long-term patency of small-diameter vascular graft made from fibroin, a silk-based biodegradable material. J Vasc Surg 2010;51:155-64
32. Altman GH, Horan RL, Lu H, et al. Silk matrix for tissue engineered anterior cruciate ligaments. Biomaterials 2002;23:4131-41
33. Choi H-M, Bide M, Phaneuf M, et al. Antibiotic treatment of silk to produce novel infection-resistant biomaterials. Text Res J 2004;74:333-42
34. Nazarov R, Jin HJ, Kaplan DL. Porous 3-D scaffolds from regenerated silk fibroin. Biomacromolecules 2004;5:718-26
35. Hardy JG, Romer LM, Scheibel TR. Polymeric materials based on silk proteins. Polymer 2008;49:4309-27
36. Wang Y, Kim H-J, Vunjak-Novakovic G, Kaplan DL. Stem cell-based tissue engineering with silk

biomaterials. Biomaterials 2006;2:6064-82

- **Review of silk fibroin material formats studied as tissue engineering scaffolds.**

37. Park S-H, Gil ES, Shi H, et al. Relationships between degradability of silk scaffolds and osteogenesis. Biomaterials 2010;31:6162-72
38. Makaya K, Terada S, Ohgo K, Asakura T. Comparative study of silk fibroin porous scaffolds derived from salt/water and sucrose/hexafluoroisopropanol in cartilage formation. J Biosci Bioeng 2009;108:68-75
39. Kim U-J, Park J, Li C, et al. Structure and properties of silk hydrogels. Biomacromolecules 2004;5:786-92
40. Tsukada M, Freddi G, Minoura N, Allara G. Preparation and application of porous silk fibroin materials. J Appl Polym Sci 1994;54:507-14
41. Guziewicz N, Best A, Perez-Ramirez B, Kaplan DL. Lyophilized silk fibroin hydrogels for the sustained local delivery of therapeutic monoclonal antibodies. Biomaterials 2011; 32:2642-5
42. Uebersax L, Mattotti M, Papaloizos M, et al. Silk fibroin matrices for the controlled release of nerve growth factor (NGF). Biomaterials 2007;28:4449-60
43. Uebersax L, Merkle HP, Meinel L. Insulin-like growth factor I releasing silk fibroin scaffolds induce chondrogenic differentiation of human mesenchymal stem cells. J Control Release 2008;127:12-21
44. Zhang X, Reagan MR, Kaplan DL. Electrospun silk biomaterial scaffolds for regenerative medicine. Adv Drug Deliv Rev 2009;61:988-1006
45. Jin H-J, Chen J, Karageorgiou V, et al. Human bone marrow stromal cell responses on electrospun silk fibroin mats. Biomaterials 2004;25:1039-47
46. Li C, Vepari C, Jin H-J, et al. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials 2006;27:3115-24
47. Lu Q, Hu X, Wang X, et al. Water-insoluble silk films with silk I structure. Acta Biomater 2010;6:1380-7
48. Jin H-J, Park J, Karageorgiou V, et al. Water-insoluble silk films with reduced beta-sheet content. Adv Funct Mater 2005;15:1241-7
49. Demura M, Asakura T, Kuroo T. Immobilization of biocatalysts with bombyx mori silk fibroin by several kinds of physical treatment and its application to glucose sensors. Biosensors 1989;4:361-72
50. Miyairi S, Sugiura M, Fukui S. Immobilized beta glucosidase in fibroin membrane. Agric Biol Chem 1978;42:1661-7
51. Acharya C, Kumar V, Sen R, Kundu SC. Performance evaluation of a silk protein-based matrix for the enzymatic conversion of tyrosine to L-DOPA. Biotechnol J 2008;3:226-33
52. Bayraktar O, Malay O, Ozgarip Y, Batigun A. Silk fibroin as a novel coating material for controlled release of theophylline. Eur J Pharm Biopharm 2005;60(3):373-81
53. Chen J, Minoura N, Tanioka A. Transport of pharmaceuticals through silk fibroin membrane. Polymer 1994;35:2853-6
54. Hines DJ, Kaplan DL. Mechanisms of controlled release from silk fibroin films. Biomacromolecules 2011; 12:804-12
55. Liu X-Y, Zhang C-C, Xu W-L, Ouyang C. Controlled release of heparin from blended polyurethane and silk fibroin film. Mater Lett 2009;63:263-5
56. Kikuchi J, Mitsui Y, Asakura T, et al. Spectroscopic investigation of tertiary fold of staphylococcal protein A to explore its engineering application. Biomaterials 1999;20:647-54
57. Zhang Y-Q, Shen W-D, Gu R-A, et al. Amperometric biosensor for uric acid based on uricase-immobilized silk fibroin membrane. Anal Chim Acta 1998;369:123-8
58. Wu Y, Shen Q, Hu S. Direct electrochemistry and electrocatalysis of heme-proteins in regenerated silk fibroin film. Anal Chim Acta 2006;558:179-86
59. Putthanarat S, Eby RK, Naik RR, et al. Nonlinear optical transmission of silk/green fluorescent protein (GFP) films. Polymer 2004;45:8451-7
60. Wang X, Hu X, Daley A, et al. Nanolayer biomaterial coatings of silk fibroin for controlled release. J Control Release 2007;121:190-9
61. Wang X, Zhang X, Castellot J, et al. Controlled release from multilayer silk biomaterial coatings to modulate vascular cell responses. Biomaterials 2008;29:894-903
62. Wang X, Wenk E, Hu X, et al. Silk coatings on PLGA and alginate microspheres for protein delivery. Biomaterials 2007;28:4161-9
63. Gobin AS, Rhea R, Newman RA, Mathur AB. Silk-fibroin-coated liposomes for long-term and targeted drug delivery. Int J Nanomed 2006;1:81-7
64. Mathur AB, Gupta V. Silk fibroin-derived nanoparticles for biomedical applications. Nanomedicine 2010;5:807-20
65. Nathwani BB, Jaffari M, Juriani AR, et al. Fabrication and characterization of silk-fibroin-coated quantum dots. IEEE Trans Nanobioscience 2009;8:72-7
66. Chang S, Dai Y, Kang B, et al. Fabrication of silk fibroin coated ZnSe:Mn2+ quantum dots under gamma-radiation and their magnetic properties. Solid State Commun 2009;149:1180-3
67. Wang X, Kim HJ, Xu P, et al. Biomaterial coatings by stepwise deposition of silk fibroin. Langmuir 2005;21:11335-41
68. Cheema SK, Gobin AS, Rhea R, et al. Silk fibroin mediated delivery of liposomal emodin to breast cancer cells. Int J Pharm 2007;341:221-9
69. Pritchard EM, Szybala C, Boison D, Kaplan DL. Silk fibroin encapsulated powder reservoirs for sustained release of adenosine. J Control Release 2010;144:159-67
70. Katayama H, Issiki M, Yoshitomi H. Application of fibroin in controlled release tablets containing theophylline. Biol Pharm Bull 2000;10:1229-34
71. Pritchard EM, Valentin T, Boison D, Kaplan DL. Incorporation of proteinase inhibitors into silk-based delivery devices for enhanced control of degradation and drug release. Biomaterials 2011;32:909-18
72. Wang X, Wenk E, Matsumoto A, et al. Silk microspheres for encapsulation and controlled release. J Control Release 2007;117:360-70
73. Hino T, Shimabayashi S, Nakai A. Silk microspheres prepared by spray-drying of an aqueous system. Pharm Pharmacol Commun 2000;6:335-9

74. Hino T, Tanimoto M, Shimabayashi S. Change in secondary structure of silk fibroin during preparation of its microspheres by spray-drying and exposure to humid atmosphere. J Colloid Interface Sci 2003;266:68-73

75. Yeo J-H, Lee K-G, Lee Y-W, Kim SY. Simple preparation and characteristics of silk fibroin microsphere. Eur Polym J 2003;39:1195-9

76. Cao Z, Chen X, Yao J, et al. The preparation of regenerated silk fibroin microspheres. Soft Matter 2007;3:910-15

77. Bessa PC, Balmayor ER, Azevedo HS, et al. Silk fibroin microparticles as carriers for delivery of human recombinant BMPs. Physical characterization and drug release. J Tissue Eng Regen Med 2010;4:349-55

78. Bessa PC, Balmayor ER, Hartinger J, et al. Silk fibroin microparticles as carries for delivery of human recombinant bone morphogenetic protein-2: in vitro and in vivo bioactivity. Tissue Eng C Methods 2010;16:937-45

79. Lammel AS, Hu X, Park S-H, et al. Controlling silk fibroin particle features for drug delivery. Biomaterials 2010;31:4583-91

80. Imsombut T, Srisuwan Y, Srihanam P, Baimark Y. Genipin-cross-linked silk fibroin microspheres prepared by the simple water-in-oil emulsion solvent diffusion method. Powder Technol 2010;203:603-8

81. Wenk E, Wandrey AJ, Merkle HP, Meinel L. Silk fibroin spheres as a platform for controlled drug delivery. J Control Release 2008;132:26-34

82. Wang X, Yucel T, Lu Q, et al. Silk nanospheres and microspheres from silk/pva blend films for drug delivery. Biomaterials 2010;31:1025-35

83. Kundu J, Chung YI, Kim YH, et al. Silk fibroin nanoparticles for cellular uptake and control release. Int J Pharm 2010;388:242-50

84. Wang X, Kluge JA, Leisk GG, Kaplan DL. Sonication-induced gelation of silk fibroin for cell encapsulation. Biomaterials 2008;29:1054-64

85. Yucel T, Cebe P, Kaplan DL. Vortex-induced injectable silk fibroin hydrogels. Biophys J 2009;97:2044-50

86. Hanawa T, Watanabe A, Tsuchiya T, et al. New oral dosage form for elderly patients. II. release behavior of benfotiamine from silk fibroin gel. Chem Pharm Bull 1995;43:872-6

87. Fang J-Y, Chen J-P, Leu Y-L, Wang H-Y. Characterization and evaluation of silk protein hydrogels for drug delivery. Chem Pharm Bull 2006;54:156-62

88. Megeed Z, Haider M, Li D, et al. In vitro and in vivo evaluation of recombinant silk-elastinlike hydrogels for cancer gene therapy. J Control Release 2004;94:433-45

89. Greish K, Araki K, Li D, et al. Silk-elastinlike protein polymer hydrogels for localized adenoviral gene therapy of head and neck tumors. Biomacromolecules 2009;10:2183-8

90. Gustafson J, Greish K, Frandsen J, et al. Silk-elastinlike recombinant polymers for gene therapy of head and neck cancer: from molecular definition to controlled gene expression. J Control Release 2009;140:256-61

91. Cappello J, Crissman JW, Crissman M, et al. In-situ self-assembling protein polymer gel systems for administration, delivery, and release of drugs. J Control Release 1998;53:105-17

92. Dinerman AA, Cappello J, Ghandehari H, Hoa SW. Solute diffusion in genetically engineered silk–elastinlike protein polymer hydrogels. J Control Release 2002;82:277-87

93. Haider M, Megeed Z, Ghandehari H. Genetically engineered polymers: status and prospects for controlled release. J Control Release 2004;95:1-26

94. Gustafson JA, Ghandehari H. Silk-elastinlike protein polymers for matrix-mediated cancer gene therapy. Adv Drug Deliv Rev 2010;62:1509-23

95. Chen J, Altman GH, Karageorgiou V, et al. Human bone marrow stromal cell and ligament fibroblast responses on RGD-modified silk fibers. J Biomed Mater Res A 2003;67:559-70

96. Meinel L, Karageorgiou V, Fajardo R, et al. Bone tissue engineering using human mesenchymal stem cells: effects of scaffold material and medium flow. Ann Biomed Eng 2004;32:112-22

97. Wongpanit P, Ueda H, Tabata Y, Rujiravanit R. In vitro and in vivo release of basic fibroblast growth factor using a silk fibroin scaffold as delivery carrier. J Biomater Sci Polym Ed 2010;21:1403-19

• **Study that investigates both diffusion- and degradation-driven drug release from a silk fibroin scaffold that functions as a cell support and directs differentiation via growth factor release.**

98. Karageorgiou V, Meinel L, Hofmann S, et al. Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells. J Biomed Mater Res A 2004;71:528-37

99. Chen B, Yin C, Cheng Y, et al. Using silk woven fabric as support for lipase immobilization: the effect of surface hydrophilicity/hydrophobicity on enzymatic activity and stability. Biomass Bioenerg 2010; In press

100. Wenk E, Murphy AR, Kaplan DL, et al. The use of sulfonated silk fibroin derivatives to control binding, delivery and potency of FGF-2 in tissue regeneration. Biomaterials 2010;31:1403-13

101. Wang X, Kaplan DL. Functionalization of silk fibroin with neutravidin and biotin. Macromol Biosci 2011;11:100-10

102. Vepari CP, Kaplan DL. Covalently immobilized enzyme gradients within three-dimensional porous scaffolds. Biotechnol Bioeng 2006;93:1130-7

103. Murphy AR, Kaplan DL. Biomedical applications of chemically-modified silk fibroin. J Mater Chem 2009;19:6443-50

104. Hardy JG, Scheibel TR. Composite materials based on silk proteins. Prog Polym Sci 2010;35:1093-115

•• **An excellent review of composite systems that incorporate silk biomaterials.**

105. Madduri S, Papaloizos M, Gander B. Trophically and topographically functionalized silk fibroin nerve conduits for guided peripheral nerve regeneration. Biomaterials 2010;31:2323-34

106. Wang X, Wenk E, Zhang X, et al. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. J Control Release 2009;134:81-90

107. Wilz A, Pritchard EM, Li T, et al. Silk polymer-based adenosine release: therapeutic potential for epilepsy. Biomaterials 2008;29:3609-16

108. Szybala C, Pritchard EM, Lusardi TA, et al. Antiepileptic effects of silk-polymer based adenosine release in kindled rats. Exp Neurol 2009;219:126-35

- **Demonstrates the therapeutic efficacy of adenosine-releasing silk brain implants and excellent agreement between in vitro release behavior and in vivo seizure suppression.**

109. Wenk E, Meinel AJ, Wildy S, et al. Microporous silk fibroin scaffolds embedding PLGA microparticles for controlled growth factor. Biomaterials 2009;30:2571-81

110. Mandal BB, Mann JK, Kundu SC. Silk fibroin/gelatin multilayered films as a model system for controlled drug release. Eur J Pharm Sci 2009;37:160-71

111. Mandal BB, Kapoor S, Kundu SC. Silk fibroin/polyacrylamide semi-interpenetrating network hydrogels for controlled drug release. Biomaterials 2009;14:2826-36

112. Kwon TK, Kim JC. Complex coacervation-controlled release from monoolein cubic phase containing silk fibroin and alginate. Biomacromolecules 2010; 12:466-71

113. Mandal BB, Kundu SC. Calcium alginate beads embedded in silk fibroin as 3D dual drug releasing scaffolds. Biomaterials 2009;30:5170-7

114. Jiang X, Zhao J, Wang S, et al. Mandibular repair in rats with premineralized silk scaffolds and BMP-2 modified bMSCs. Biomaterials 2009;30:4522-32

115. Uebersax L, Fedele DE, Schumacher C, et al. The support of adenosine release from adenosine kinase deficient ES cells by silk substrates. Biomaterials 2006;27:4599-607

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