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Silk constructs for delivery of musculoskeletal therapeutics

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Abstract

Silk fibroin (SF) is a biopolymer with distinguishing features from many other bio- as well as synthetic polymers. From a biomechanical and drug delivery perspective, SF combines remarkable versatility for scaffolding (solid implants, hydrogels, threads, solutions), with advanced mechanical properties and good stabilization and controlled delivery of entrapped protein and small molecule drugs, respectively. It is this combination of mechanical and pharmaceutical features which render SF so exciting for biomedical applications. Its pattern along with the versatility of this biopolymer have been translated into progress for musculoskeletal applications. We review the use and potential of silk fibroin for systemic and localized delivery of therapeutics in diseases affecting the musculoskeletal system. We also present future directions for this biopolymer as well as the necessary research and development steps for their achievement.

Keywords

silk-fibroin; musculoskeletal diseases; drug delivery; implant; injectable drug delivery systems

Introduction to silk

Sericulture (silk farming) has its origin in China about 5,000 years ago and silk was the subject of intensive trading with the west [1]. Two structural proteins, silk fibroin (SF) heavy chain (~ 370 kDa) and light chain (~ 25 kDa) are held together by sericin proteins that act with glue like functions [2-4]. Sericins – hydrophilic proteins - have been linked to immunogenic reactions of silk - whereas SFs have not - and can be easily separated from SF by extraction in alkaline solutions [5-8]. Heavy chain SF is characterized by a series of alternating hydrophobic and hydrophilic domains (this is why it is regularly referred to as nature's equivalent to block polymers), rendering the molecule amphiphilic. During solidification, SF forms β -sheet crystalline structures and by virtue of interacting hydrophobic domains consisting of repeats rich in glycine and alanine. Almost all manufacturing protocols to date isolate SF from whole silk (cocoons) by boiling in Na_2CO_3 solution [6, 7, 9]. In addition to sericulture sources, genetically engineered SF analogues have become available [10], low MW analogues to SF. Genetically engineered SF analogues

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may allow the effective deployment of certain aspects of SF for advanced, bioengineered polymers. Cost of goods for such genetically engineered supplements may be an issue, which may challenge their broader use unless the constructs contain pharmacological action (e.g. bioconjugates), such that these can be positioned as drugs rather than supplemental excipients.

The solubility of SF is low in most common organic solvents and dissolution occurs in chaotropic solutions with high salt concentrations required to destabilize the β -sheet [11]. The next processing step involves removal of the electrolytes and either lyophilization (which requires dissolution in organic solvents such as hexafluoroisopropanol) or continuous processing of the aqueous solution [6, 7]. A general caveat for SF solutions at high concentrations is avoiding premature β -sheet formation and aggregation. These processes can be controlled by solution composition or via SF surface decoration with acidic groups to increase inter-molecular repulsive forces and early results are encouraging [12]. In addition, ionic liquids proved successful in balancing the system against premature β -sheet formation [11]. From a processing perspective, SF is a stable protein and autoclaving to obtain sterility can be done without substantially impacting physical properties [13]. Toxicological evaluations of SF have been encouraging, placing foreign body reactions following the implantation of SF films comparable to those observed with type I collagen films and less than what was observed for films made from polylactide-co-glycolide (PLGA) synthetic polymer, which is a slowly degrading polymer used in the pharmaceutical market for controlled drug delivery [14-16].

Generalized musculoskeletal diseases – how can SF contribute to better medication?

Disorders of bone and cartilage can be divided into either generalized or localized disorders. The treatment of either category requires rather different specification to a drug or drug conjugate, particularly from a pharmacokinetic (PK) perspective. Many congenital or acquired disorders to bone are at least in part of genetic cause such as enzymatic defects, or caused by toxic agents during intrauterine life or later. For example, Hypophosphatasia (HPP) is a metabolic disorder and clinical symptoms including poorly mineralized bones are caused by deficient tissue nonspecific alkaline phosphatase (TNSALP) in osteoblasts and chondrocytes. Patients respond favorably to systemic enzyme replacement therapy (ERT) [17] and when the enzyme is presented on appropriate scaffolds, such as when fused to the Fc part of a monoclonal human antibody and tagged with reported bone-targeting moieties by means of short poly-aspartic acid sequences [18]. PK patterns of this enzyme-Fc fusion protein are governed by the Fc part being bound by the neonatal Fc receptor (nFcR), resulting in half lives of several days to weeks allowing extended replenishment of lost enzyme activity during ERT. SF-drug conjugates may offer novel approaches to systemic administration and presentation of such therapeutics. The systemic use of such conjugates has only rarely been investigated in relevant animal model systems so far and to our knowledge not at all in clinical studies. Therefore, questions remain unanswered regarding the use of SF in bioconjugates, particularly regarding possible immune responses by either classical activation of the immune system or breaking B cell tolerance by an SF-human protein bioconjugate. The classical activation is a function of non-self epitope presentation. SF is not a mammalian protein and from that perspective one would expect an immune response, particularly after subcutaneous administration [19]. In spite of this consideration, studies reported so far do not suggest a strong immunogenic potential for SF. For example, SF-insulin bioconjugates, which were synthesized by means of glutaraldehyde cross-linking resulted in favorable outcome [20]. In these studies, SF alone was assessed for immunogenicity in rabbits (intravenous administration) and the SF-insulin bioconjugates assessed for antigenicity in rats (intramuscular administration). The authors reported that the

bioconjugates demonstrated substantially prolonged efficacy as compared to insulin alone and absence of immunogenic reactions to SF alone. No antibodies were formed against insulin when presented as part of the bioconjugate. In another study, in which the authors presented SF-asparaginase bioconjugates, similar encouraging results were reported. Asparaginase is known to be immunogenic and conjugation with polymers such as PEG resulted in reduced antigenicity when administered intramuscularly [21]. In these studies of SF-asparaginase bioconjugates, intraperitoneal injection of SF alone proved biocompatible when dissolved in aqueous vehicle [22]. In addition to these important immunological studies, the SF-asparaginase bioconjugate was found to stabilize the enzyme during storage and against enzymatic break-down and decreased the immunogenicity and antigenicity of the enzyme in rabbits. These studies concluded the safe use of SF bioconjugates and provide evidence, that the systemic use of SF-drug bioconjugates may be well tolerated. Other required information before the safe use of such bioconjugates can be concluded to include information on the effect of length of treatment, the impact of the administration route (particularly the effect of subcutaneous administration on immune responses), or the presence of impurities on safety parameters [23]. Potential application of such systems for musculoskeletal application may be in enzyme replacement therapy (ERT), an application within which any antibody formation against the active part of a bioconjugate would be particularly threatening. Such an antibody response places patients at risk that their perhaps reduced enzymatic activity may be further challenged in presence of such antibodies formed in response to presentation of enzyme epitopes as part of the bioconjugate structure. Future studies must detail if SF as part of bioconjugates can help to suppress such antibody responses to the active part and, thereby, help to address a major threat in ESR. Another consideration in ESR is the efficient presentation of the therapeutic enzyme to the mostly affected organ or tissue. For that, such enzymes can e.g. be entrapped in particulate systems or – as presented above – as soluble bioconjugates. For example, Morbus Gaucher – a disease leading to multiple morbidities including skeletal manifestation – is a lysosomal storage disease (LSD) with the liver being the mainly affected organ and characterized by the accumulation of glucosylceramide in macrophages resulting in their activation [24]. Targeting of liver tissue may be more easily achieved with particulate systems [25] carrying the replacing enzyme. Such particles – if left undecorated – are likely cleared by liver residing macrophages, or Kupffer cells. Thereby, organ and cell targeting may be achieved with particles, as is efficient uptake into the affected lysosomal compartment. Such general strategies have been reviewed before, some of which may be successfully transferred to SF [26]. In contrast, Morbus Pompe another LSD requires efficient delivery of the replacing enzyme to the skeletal muscle, a task which is arguably more successful with (dissolved) bioconjugates rather than with particulate systems. It will be exciting to follow if the initial evidence for the well tolerated SF in bioconjugates will be corroborated for other drugs / enzyme bioconjugates and provide a wider path of development along these lines.

Other generalized diseases to bone include osteoporosis (low bone matrix), osteopetrosis (disturbed architecture), osteitis deformans (disturbed architecture and mineralization) or osteitis fibrosa (low bone matrix, disturbed architecture and mineralization) among numerous other disorders affecting bone structure. For example, osteoporosis leads to increased fracture risk particularly at the hip, spine and wrist, which accounted for 297,000 hip fractures, 547,000 vertebral fractures, 397,000 wrist fractures, 135,000 pelvic fractures and 675,000 fractures at other sites in the US alone in 2005. Osteoporosis is the main cause for all femoral neck fractures [27]. More recently, reduction of catabolic target molecules such as RANK-L [28] or M-CSF [29] have found clinical interest to treat osteoporosis, such that osteoclastic activity and bone resorption are reduced. Similarly, the removal of factors negatively controlling the Wnt signaling pathway in osteoblasts such as sclerostin and members of the DKK family of proteins forms an exciting therapeutic pathway which is currently explored in clinical studies [30]. Indeed, sclerostin depletion by means of

humanized monoclonal sclerostin antibody, has established as a powerful approach to favorably modulate bone resorption in humans leading to an increase in bone mineral density in both, the lumbar spine and total hip [31]. The common theme is that reduction of these targets is followed by a net gain in bone mass. This reduction of the target protein is typically addressed by means of administration of anti-target monoclonal antibodies, a therapy providing a sink for the target molecules. The so far recorded promising risk-benefit ratio for systemically administered SF bioconjugates (see above) or extravasally placed SF implants, films, or particles [25, 32, 33] would encourage the design of such sink using SF as a biopolymer. For example, extravasally placed, nanoscale SF particulate systems, which can be easily decorated with target binding sites [34, 35], would be placed extravasally where they would act as a sink for the targets. This approach may be challenged by the fact that it remains an open question how for example the target protein sclerostin, a 24 kDa protein, is transported through membranes / blood capillaries to efficiently enter the extravascular space as a prerequisite to interact with such an extravasally placed sink. In essence, many translational questions remain open before an assessment of these approaches for the removal of systemically circulating factors using SF systems can be seriously approached – however, as the “sink” approach is perhaps one of the major emerging therapies in musculoskeletal disease resting on antibody therapy today, it may be worth exploring the use of SF as an alternative as outlined above.

A new use of SF for systemic drug delivery has been recently described, within which SF microneedles facilitate parenteral delivery through the skin. The system – in analogy to previous systems prepared from polylactic-co-glycolic polymers (the use of which is limited by requiring high melting temperatures during manufacture [36]), steel needles coated with carboxymethylcellulose, poloxamer, trehalose blends containing the drug (the use of which is associated with the risk of breaking steel needles remaining in the skin [37]) or polyvinylpyrrolidone / carbohydrate bulk systems (which display biocompatibility but typically require curing with ultraviolet light during manufacture potentially impacting the stability of loaded drugs) - may combine the excellent stabilization properties of SF for sensitive chemical entities and biomolecules, the mechanical properties of SF allowing efficient penetration through the upper skin layer with the potential for sustained and controlled release profiles for many drugs [38]. Microneedles are short and in spite of the disruption of the skin as a prerequisite to for efficient systemic availability, their use is pain free [39]. These needles disrupt the upper, lipophilic dead skin layers and shuttle the drug into deeper skin layers such as the epidermis or perhaps upper parts of the dermis, from which the molecules are distributed systemically. It is this physical disruption of the upper skin barrier which allows the efficient delivery of hydrophilic drugs, including proteins. In principal, these needles can be either coated with the drug in which the needle only serves for penetration and as a releasing surface of the adsorbed drug or the needles can break and remain in the skin, with the needles disintegrating slowly and thereby releasing an entrapped drug in a sustained fashion. In the latter case, SF might be an interesting choice and in light of its excellent stabilization potential for protein drugs after implantation [40, 41], such that sustained delivery profiles even for sensitive drugs may become feasible by this painless approach. Studies using SF as dermal fillers would suggest such intradermally placed needles and, thereby, the sustained presence within the skin will be biocompatible [42]. Release kinetics from such SF needles may be tailored by post-processing treatment as a function of system crystallinity, as has been demonstrated for SF particles, films or scaffolds for enzymes, growth factors and other drugs, respectively [32, 35, 43-45]. A candidate drug for such SF microneedle systems within a musculoskeletal context and for which the feasibility of SF encapsulation has been demonstrated is insulin-like growth factor I (IGF-I), a factor or derivatives thereof which have potential for the treatment of osteoporotic or sarcopenic states [46, 47]. It will be exciting to follow the development of SF based microneedle systems for musculoskeletal application. Alternatively, a rapid release may

sometimes be warranted for desired pharmacological response. In these cases, the depot approach may not be advisable and the SF microneedle system would need to be engineered such that the needles do not break and the drug of choice is adsorbed to the surface and readily released after the system has been placed on the skin. Within the context of musculoskeletal diseases, a particularly successful approach for such a micro-needle delivery system is with parathyroid hormone fragment 1-34 (PTH₁₋₃₄) [48-50]. The pharmacokinetic challenge of PTH therapeutics is the requirement for intermittent delivery as prolonged plasma profiles favor osteoclastic resorption over osteoblastic bone formation and thereby, catabolic responses whereas intermittent PTH delivery results in stronger anabolic than catabolic responses and, thereby, an overall net gain in bone mass [30]. SF by virtue of the fact that it is a relatively acidic macromolecule absorbs most growth factors, enzymes and other therapeutic proteins efficiently, such that surface adsorption on SF needles should be readily amenable including PTH₁₋₃₄ which has a pI of around 9 [2, 45, 50-52] followed by rapid PTH release once inserted into the skin [48]. Thereby, short pulses of PTH delivery may become feasible, providing rapid release from PTH₁₋₃₄ coated needles is readily achieved after a patch has been placed.

Localized musculoskeletal diseases – how can SF contribute to better medication?

Infection

Localized disorders for musculoskeletal diseases include infection, typically requiring systemic therapy or – when resulting from surgery – localized treatment or preventive treatment in cases in which an infection must be avoided. Numerous studies support the feasibility of SF for localized delivery of chemical entities, including antibiotics such as doxycycline [53, 54], rifampicin, erythromycin [55], or tetracycline (unpublished results). Known instabilities of these drugs were overcome by entrapment within silk films or 3D scaffold material e.g. against temperature stress and sustained potency was demonstrated when antibiotics were presented from sponges and as compared to presentation of the (unprotected) drugs in solution. It is this combination of a stabilizing effect along with the ability to deliver for sustained periods and interesting mechanical properties for such implants in a bone defect (for review of mechanical properties, see e.g. [56, 57]) that renders the use of SF particularly interesting for application as bone defect fillers releasing antibiotics to prevent post-operative infection.

Tumors

Tumors are quite prominent in bone as compared to many other sites of the body and bone tissue is a frequent site of metastatic infiltration. Efficient delivery has been demonstrated for several chemotherapeutics from SF or SF composites with other (bio-) polymers. Recently, an exciting development of SF based drug delivery systems for the repair of tumor resection sites has been described. Emodin – a rather hydrophobic anthraquinone with anti-cancer potential – delivery from liposomes embedded within SF / chitosan scaffolds resulted in successfully “reduced tumor presence, scaffold degradation, remodeling, and new tissue deposition” [58] when tested for breast cancer therapy using a rat model. This work is relevant for musculoskeletal application, as it may lead to the combination of the demonstrated mechanical and biocompatibility advantages of SF scaffolds as bone filler or for fracture repair and reconstruction [59] as has been demonstrated when seeded with human mesenchymal stem cells [60, 61] or when ectopically grafted periosteum was used as cell source [62] with the opportunity to provide a sustained release of an anti-cancer drug which may be a small molecule such as emodin [58], a biopharmaceutical (feasibility demonstrated for non-cancer therapy [32, 45]) or by means of gene delivery (feasibility demonstrated for non-cancer therapy [63]). Within the musculoskeletal context, the

advantages of SF may be particularly valuable in a rare disease affecting the larger joints - pigmented villonodular synovitis (PVNS). The etiology of PVNS has more recently been linked to a translocation of the colony stimulating factor I (CSF-1) gene under control of the collagen type 6 promotor in a small number of cells, leading to infiltration of inflammatory cells and a prominent synovitis leading to bone erosion and lysis in advanced stages [64, 65]. PVNS is treated by radical synovectomy. PVNS patients are typically around 20 – 50 years of age and healthy other than the affected index joint. Treatment is by synovectomy and in advanced stages by additional removal of affected bone leading to joint replacement in particularly severe cases. Clinical success is quite frequently challenged by recurrence as a result of incomplete synovectomy and may be addressed where indicated by radiation synovectomy. Potentially, drug loaded SF scaffolds may serve as an interesting filler of such defects. In addition to their support function and as filler of the removed bone or when placed into the affected joints they may provide sustained drug delivery of drugs to perhaps serve as a scaffold to open pharmacological alternatives to surgery to PVNS patients or avoid or delay relapse following surgery. Such drugs may include antibodies to CSF-1 or perhaps small molecules such as the tyrosine kinase inhibitor imatinib, which has demonstrated success in PVNS therapy [66]. Aside from using three dimensional fillers of SF, the use of injectable SF hydrogels may be interesting as outlined below [67-72]. Therefore, PVNS may be a good translational indication to move SF forward in context of localized tumor treatment, before expanding into broader indications for which success criteria may be more difficult to assess.

Localized bone repair with solid SF implants

Local implantation of a scaffold material is frequently motivated by (re-)generating tissue function with the implant being gradually replaced by self repair. This typically requires adjusted implant degradation rates, matching the rate of tissue formation. For silk-fibroin (SF), adjustments of the degradation rates have mostly been achieved by coating of solid SF with hydroxyapatite [73], or blending of polymers [74], among others strategies. However, recent insight has provided important clues to optimization routes towards improved material outcome and the critical relationship between degradability of silk scaffolds and osteogenesis has been reviewed before [75-78]. SF, after isolation from cocoons or from other sources, is a water soluble material as are implants cast from SF. Only after exposure to water vapor or methanol or other suitable organic solvents, SF organizes into β -sheet structures (referred to as silk II) and it is this conformational rearrangement which renders the material water insoluble [6, 7, 79-82]. Consequently, the efficacy of SF transformation into β -sheet structures is regarded as a measure to tailor degradation rates, mechanical properties, or – within the context of SF-drug composites, release rates of therapeutics entrapped in the biopolymer [25, 35, 83, 84]. However, recent insights challenge this arguably simplistic perspective following the finding that water *insoluble* scaffolds predominantly arranged in silk I crystal structure – an intermediate crystal form regarded as a precursor form required for efficient transformation into silk II crystals in biological alignment processes within spider or insect glands [75, 79, 85]. These silk I films were more flexible and degraded faster as compared to silk II films, thereby expanding the accessible window for tailored mechanical properties, degradation patterns and associated drug release kinetics. The current model of SF degradation describes, that initially the first structures removed from the lattice in both, PBS and protease solutions are non-crystalline hydrophilic domains, followed by silk I and ultimately silk II elements. First modeling suggested, that the disintegration of the non-crystalline and silk I structures lead to a gradual destabilization of the silk material or scaffold such that as a result of break-down, crystalline particles are released from the bulk [86]. Furthermore, the amount of particle release was a function of manufacturing conditions, which impact the network composition of the resulting scaffold material [87]. Tailored particle release and adjusted degradation kinetics may form valuable

optimization parameters to meet clinical requirements and adjust toxicological profiles in the future. In brief, degradation rate and element crystallinity are inversely related [88-90].

Mechanical properties of SF itself have supported successful applications in preclinical studies resulting in tissue repair and including load bearing applications as an implant in rat femoral critical size defects [40, 91]. Comparable properties have been found for scaffolds from aqueous based manufacturing protocols [92-94] as well as those deploying organic solvents [60, 95]. Nevertheless, surgical interventions using these implants at load bearing sites required plate-fixation, as the scaffolds themselves provide insufficient mechanical support and would collapse under the impact of body weight and muscle function [40, 60, 91]. Building off these experiments SF scaffolds were engineered with the goal to provide scaffolds with compressive modulus on par with those of trabecular bone (ranging from about 10 to 50 MPa [96, 97]). First attempts included the simple immersion of SF scaffolds into CaCl_2 / Na_2HPO_4 solutions, a surface modifying process which failed to substantially increase the properties of the non-soaked scaffolds [73, 98]. Later protocols were reported to yield in better mechanical properties but resulted in small pore diameters which were less suitable for application as a bone substituting material [99]. Another promising approach to increase the mechanical properties of silks has been the addition of silk particles during scaffold preparation [100], which has recently been refined [99]. Apart from addressing insufficient material stiffness by co-formulation of particles these protocols offer an exciting opportunity at the implant / drug delivery interface, as drugs and drug release can be conveniently tailored using SF spheres [32, 45]. The combination of these two properties must be addressed in future studies.

Silks are the strongest natural fibrous materials known and even rival the best synthetic high performance fibers such as Kevlar in terms of energy absorbed to break [101] which sparked interest of this material for ligament replacement since centuries [102-104] and has more recently resulted in clinical studies on anterior cruciate ligament (complete ACL rupture) replacement [105, 106]. This medical device has the advantage that the native mechanical parameters of silks were directly translated into the resulting ligament implant and did not require preceding dissolution of the silk structure as most published systems do [95, 107-109]. Therefore, these ligaments contain all elements of native silk and are not isolating silk fibroin prior to manufacture. Isolation of silk fibroin comes at the price of destruction of the precise fiber assembly present in untreated silk and is currently preventing the exploitation of the astonishing variability of natural silks for biomedical applications. For example, silks are used in nature for such diverse applications as cocoon fabrication (*Bombyx mori*) or in underwater processes resulting in non-water soluble structures such as reservoir formation to trap air bubbles for breathing (*Agyronetidae*) or prey wrapping of nets (*Polycentropidae*, *Psychosomiidae*, *Hydropsychidae*, *Agyronetidae*) [110, 111] and in all these cases the building blocks are adjusted to meet environmental specifics with astonishing perfection. Current protocols aiming at the isolated use of SF are unable to restore this fine control nature provides and in spite of the fact that it is unclear how this hurdle may be overcome, nature's variability indicates the broad range of functions which can be covered by silks and perhaps isolated SF. The combination of ligament engineering and drug delivery has been recently presented [112].

SF properties can be tailored by blending with other polymers to improve handling properties during scaffold manufacture and tailor mechanical properties and / or architecture of the resulting implant materials. Nevertheless, from a pharmaceutical perspective, these blending properties provide an interesting platform to optimize the scaffold for drug release, either by allowing (i) better stability profiles during storage and after implantation, by (ii) preventing phase separation of the drug and the biopolymer blend – a frequent cause of inhomogeneous drug release profiles – or (iii) by increasing the drug load of and drug

distribution within the scaffold material, respectively. Some successful blending approaches have been described including blends of SF with polyvinylalcohol (PVA) [34], polyethyleneoxide [113], glycerol [114] or hyaluronic acid [74, 115]. For example, blending with glycerol would be expected to partly replace water at the SF polymer and drug interface, putatively increasing resistance of drugs against hydrolysis. From a thermodynamic perspective, the blending may help to impact the interaction of protein drugs with solutes and stabilize the native form rather than its unfolded form through chemical potential increase of the unfolded form over the native state driven by surface area differences and leading to different free energy levels [116, 117].

Excellent loading of protein therapeutics into SF, including insulin like growth factor I (IGF-I), VEGF, BMPs, fibroblast growth factor, nerve growth factor and others has been demonstrated [25, 32, 35, 40, 44, 118-120]. The loading with small molecules has been linked to the drug's pKa and pI [35]. For example, salicylic acid (more negatively charged at neutral pH over SF) was rapidly released from SF and demonstrated low binding, whereas propranolol (more positively charged at neutral pH and a hydrophobic base) was bound tighter as indicated by a more sustained release profile and overall loading efficacy. These studies demonstrated, that as with any other excipient, SF based formulations require individual development for optimized, pharmaceutical outcome and drug delivery. However, the field would clearly benefit from future in vivo studies within which not only efficacy but also pharmacokinetic parameters are addressed. These are essential for the evaluation of suitability, particularly in balancing protein drug binding to SF during manufacture with bioavailability following implantation / administration. SF polymer surface decoration can be used to fine-tune this balance. In a recent study, fibroblast growth factor 2 (FGF2) has been used as a model therapeutic protein. SF surfaces were negatively charged with different surface densities by introducing sulfonic acid groups through covalent modification of the biopolymer's backbone [12, 52, 121]. The binding and release of FGF2 could be tailored as a function of negative charge density, thereby allowing an easy modification of the SF for improved FGF2 loading efficacy and release kinetics.

Engineering of osteochondral composite tissues with SF scaffolds

The broad stabilization potential and platform versatility of SF is reflected in recent studies, within which the engineering of osteochondral grafts was attempted. Scaffolds presenting individual zones of different growth factors were designed such that each zone either set a chondrogenic or osteogenic stimulus to human mesenchymal stem cells (hMSC) seeded on such scaffolds. The hypothesis was that these hMSC can deposit cartilage- and bone-like tissues on one construct, respectively, thereby generating a composite tissue. This research was motivated by the clinical challenge of osteochondral lesions (osteoarthritis) and the inherent, poor healing capacity of these defects [122-124]. Current approaches to treat osteochondral defects include autologous cartilage harvested from non load bearing sites and inserted into the compromised site with good long-term outcome, an approach compromised by the need for second site harvest and associated potential morbidity and pain [125, 126]. Alternative approaches using cell based (e.g. MACI) or more conventional approaches (e.g. microfracturing) have several shortcomings, most importantly missing healing stimuli rendering them non-curative and resulting in progressive cartilage break down with joint replacement as clinical endpoint. The engineering and clinical use of osteochondral plugs may perhaps result in intimately connected tissues which may impact clinical outcome by means of improved 'implant-bone' to 'host-bone' and 'implant-cartilage' to 'host-cartilage' integration, respectively [127]. Conventional tissue engineering approaches provide one tissue type for regeneration or separately engineer two tissue types which are subsequently sutured together, approaches which are associated with mechanical and probably biological challenges following implantation. Recently promising evidence was obtained that the

engineering of systems within which bone and cartilage tissue can be formed in parallel form human mesenchymal stem cells have been demonstrated for SF. In recent pilot experiments (Figure 1), transforming growth factor (TGF) β 3 and insulin-like growth factor I (IGF-I) were blended (IGF-I / TGF β 3 blend defined a chondrogenic zone) or bone morphogenetic protein (BMP-2 defined an osteogenic zone) into different areas on *one* SF scaffold by electrospinning (Figure 1A, B). hMSCs seeded on these scaffolds were expected to respond differently when either seeded on an osteogenic *or* a chondrogenic substrate and cultured in separate wells. Cell culture was conducted in a specialized medium found to support both, chondrogenic and / or osteogenic differentiation of hMSC, respectively. Energy consumption as assessed by glucose content in the medium after 2 days of culture was found to correlate to GF load and tissue outcome (Figure 1C, D). High glucose consumption (Figure 1D) was associated with chondrogenic responses, which were demonstrated histologically (data not shown) and increased GAG deposition in (Figure 1F) whereas glucose consumption associated with osteogenic processes was substantially less (Figure 1C, E). This is likely reflecting the role of glucose as an energy source for building GAGs [128] along with increased glucose uptake in presence of IGF-I. Cells exposed to different stimuli responded differently in terms of calcium deposition (Figure 1E) and glycosaminoglycan (GAG) deposition (Figure 1F), respectively. Notably, chondrogenic responses were not entirely pure, and hMSC deposition of GAG was always accompanied by calcification (Figure 1E, F). In contrast, BMP-2 and BMP-2/TGF- β 3 loaded disks resulted in relatively pure mineralization and minimal chondrogenic response. In conclusion, these pilot experiments point to an SF system efficient of inducing cartilage- and bone like responses on *one* SF scaffold. Preliminary interpretation may include, that IGF-I was critical to skew the system into the chondrogenic lineage (comparison of the BMB-2 vs. BMB2/IGF-I group, within which pure osteogenic responses were skewed into chondrogenic responses by IGF-I presence as indicated by GAG increase) and IGF-I alone was unable to induce chondrogenic responses in absence of other factors (data not shown) suggesting a critical modulator role for this protein for chondrogenic outcome. Glucose consumption was higher for chondrogenic responses, a finding which may serve as a new starting point for the development of new culture media aiming to optimize energy supply for tissue outcome (Figure 1) [129]. Open questions include the performance of the system when such different growth factor loaded disks are grown in one well, scale up and development of the currently used electrospun mats to truly three-dimensional implants and ultimately assessment of *in vivo* responses in osteochondral lesions when exposed to such osteochondral implants.

Localized bone repair with particulate systems

SF has been processed successfully into nano- and microparticles resulting in biocompatible and mechanically robust drug carriers [32-34, 130-132]. The biological impact of these systems for musculoskeletal indications has been demonstrated [35]. IGF-I – a growth factor anabolic to bone [133] and cartilage tissue [44] – was encapsulated into these systems with high yield (~ 100%) and nearly zero order release profiles were recorded for up to 50 days. IGF-I delivered from SF scaffolds - which were exposed to 37°C and constantly immersed in culture medium - was still bioactive after 7 weeks, demonstrating the excellent stabilizing effect of SF particles for protein drugs. The impact of these finding on skeletal application is important, as this evidence may support a development of SF spheres for intraarticular, long-term drug release. A conventional challenge of many microparticulate systems is the initial burst of therapeutic protein within the first days, which may prevent a system's use due to toxicological challenge by the initial burst. For example, IGF-I microspheres from polylactide-co-glycolide (PLGA) synthetic polymers were tested, and initial burst release was reported to be 20% of the entire load or higher [133-136]. In contrast, SF spheres holding IGF-I did not demonstrate significant initial burst release, likely due to the inherent adsorption of IGF-I to SF through hydrophobic interaction [44, 45]. Another major

challenge with PLGA based microparticles is needle clogging challenging injection suitability. PLGA particle swelling occurs when exposed to aqueous systems and likely this swelling leads to particle adherence and ultimately clogging of syringe needles. No publication reports on syringability of SF particles, however in light of low SF swelling in water one would expect better handling properties as compared to PLGA particles e.g. translating into facilitated minimally invasive intraarticular administration of SF spheres. The reduction of swelling when exposed to water by SF was used to limit that effect when using liposomes. Liposomes were coated with SF, which suppressed the swelling observed of non-coated liposomes and thereby changed the release from swelling /diffusion driven to purely diffusional processes probably as a result of steric hindrance due to the SF coating [137]. Finally, as SF degradation does not create acidic conditions in vicinity of the degrading bulk and in contrast to PLGA microspheres, this may reduce irritation within joints or other sites, within which particles are placed or degradation of acid-labile drugs during release. These examples demonstrate that SF may help to design novel microparticle platforms for intraarticular use, to overcome some of the obstacles associated with the use of synthetic PLGA polymer systems which currently dominate this indication.

Drug load and targeting of such SF particles may be achieved by tailored particle size and optimized surface modification. SF particles can cover a broad range of particle diameters and allow easy surface modification due to the presence of functional groups including amines, alcohol groups, phenols, carboxyl groups and thiols resulting in various suitable coupling techniques for decoration. Thereby, successful functionalization included the introduction of charges [12, 52, 121, 138-140], small peptide sequences [15, 95, 136, 141, 142], sugars [143, 144], and anabolic proteins [20, 119]. Charge impacts growth factor localization and release, a finding which has been reviewed in a biomimetic context recently [2]. For example heparin sulfate proteoglycans (HSPG) have been demonstrated to impact cellular and growth factor function [145]. This interaction is critically regulated by the HSPGs sulfation pattern and alteration in sulfation of HSPG impacted FGF2 response as observed in rat mandibular condyles [146, 147]. This observation has been translated into biomedical application for FGF2 delivery, wherein which FGF2 was adsorbed to SF films carrying different surface charge profiles [52]. FGF2 activity to human mesenchymal stem cells was strongly linked to charge presentation. Another way to tune surface charge of SF particles and interaction with loaded growth factors is by blending with polysaccharides, as has been demonstrated for hyaluronic acid [74, 115], heparin [148] or chitosan [149].

Localized repair using injectable SF systems

Injectable, in situ forming drug delivery systems combine several advantages, including minimally invasive application, sustained delivery of therapeutics, perhaps reduced drug exposure while maintaining therapeutic effects, reduced generalized side effects, and improved patient compliance [150]. Current injectable systems are often based on PLGA dissolved in an organic solvent and blended with a drug in solution or suspension. These systems demonstrate an established safety and biodegradability profile and are typically offering an up to 3-6 months release window following administration (specialized systems offer even longer release windows), with low viscosity (allowing the administration with needles small in diameter) and high cost of goods in spite of the potential for end sterilization using γ -radiation if compatible for the respective system under development. The polymer of these polymer-drug blends is liquid when injected and becomes viscous and/or solid within minutes or hours after administration. Following administration of PLGA injectable drug delivery systems, the organic solvent within which the polymer was dissolved diffuses from the site of administration followed by PLGA precipitation and drug entrapment, accordingly. The lag time required for depot formation may result in burst releases until the depot has thoroughly formed. For some well tolerated drugs, these high

burst releases may be considered as clinically irrelevant and one may conclude that therefore, control if these initial burst is less of a constraint. However, the manufacturer has to take into account, that frequently quality control may nevertheless demand tight specifications for the initial burst which may become part of release specifications. Thereby, reproducible control of the initial burst is vital for a successful development of such systems. These reasons along with complex manufacturing requirements and relatively high COGS for PLGA based systems are perhaps contributing to the paucity of systems which successfully found market authorization to date. Alternative systems rest basically on five principle strategies to achieve solidification upon injection (Table 1): (i) thermoplastic pastes [151-156], (ii) in situ cross linked systems [157-162], (iii) in situ precipitation [163-167], and (iv) thermally induced gels [168-171]. Other approaches (v) exploit the natural gelification property of a protein therapeutic (e.g., Somatuline Autogel) with a general constraint that such approaches may impact absolute bioavailability of the therapeutic as some of the drug is used for drug depot formation rather than pharmacological action. Gels formed from hybrid polymers are exploiting natural assembly processes known from proteins and undergo, for example, conformational transition of the coiled-coil protein domain upon injection [172, 173]. Principally, SF shares many similarities with gels from hybrid polymers, although intra- and intermolecular organization is different (coiled coil in the hybrid polymers, β -sheet for SF). Therefore, the different principle of solidification (injection of water soluble silk I/polymer blends, rapid solidification to water insoluble silk II structure during or following injection) [79, 174-177] may offer more rapid solidification due to the immediate assembly process (as it occurs in spiders and insects in milliseconds when a web or cocoon is webbed) and may thereby help to further address the initial burst challenge intrinsically associated with the PLGA based or many other injectable systems (Table 1). Furthermore, the SF based process can be designed to be all water based facilitating filtration as well as mixing of protein therapeutics [44]. This aqueous processing renders SF principally superior to approaches, within which organic solvents are indispensable [133, 134, 178]. Other advantages reside around the benign nature of SF as compared to many other synthetic polymers and even as compared to type I collagen [8, 15, 179]. SF is completely biodegradable with the time for complete removal being impacted by the manufacturing process and ranging from 2-6 months or more than one year [16]. The natural assembly process of silk includes a continuous increasing concentration of the protein blend within the spinneret by active water removal and modulation of factors such as ionic strength, pH and various ion concentrations to prevent premature aggregation. The ultimate solidification is critically initiated at the spinneret's outlet, at which by means of its rapid striation mechanical stress is induced resulting in instantaneous solidification into silk threads [4, 79, 175, 176]. In terms of sol-gel transition, a secondary structural change is causal to transform a disordered state into β -sheet-rich conformation, with solution pH, SF concentration and processing temperature being important input parameters for tailored transition kinetics [180, 181]. These insights into natural silk processing were used for the design of advanced manufacturing protocols for injectable SF depot systems. Following such a biomimetic approach, the striation in insects and spiders as the last critical natural 'processing' step inducing silk solidification was imitated by ultrasonication. By modulating sonication parameters (mimicking the striation frequency in nature), SF concentration, pH and potassium concentration, gelation was controlled between minutes to hours. Mechanistically, ultrasonication accelerated the formation of identical conformational transitions as occur with time and, thereby, sonication can be regarded as a very interesting method to initiate gel setting within seconds [72, 182, 183]. This approach required additional instrumentation for injection, e.g. special needles within which the SF / drug bulk solution was exposed to sonication during injection. The success of this approach was reported in a recent preclinical proof of concept trial on a skeletal indication, with vascular endothelial growth factor (VEGF) and bone morphogenic protein (BMP) being administered within an injectable SF gel. The sol-gel transition of this loaded gel was initiated by

sonication [72]. Growth factor (GF) release was followed for 28 days and was reported to have 'no obvious burst release' [72] and release was continuing throughout the monitored period during which the GFs demonstrated bioactivity. VEGF and BMP-2 release from this system successfully induced angiogenesis and bone formation indicating an excellent activity with good distribution of the drug within the irregular bony cavities. Recently, a novel approach has been developed, within which sonication was replaced by vortex formation for rapid induction of β -sheet structures / sol-gel transition with minimal instrumental requirements as required for the ultrasonication approach. These studies started off from the observation, that aqueous silk solutions undergo a transition from states initially rich in random coil to one rich in β -sheet content in response to shear stress as occurring during vortex formation [71, 184]. The approach resulted in rapid hydrogelation kinetics and may form another exciting mechanism to induce sol-gel transition in SF injectable depot systems. Future experiments have to detail the application of SF for the design of injectable drug delivery systems for musculoskeletal diseases and Table 2 details challenges for future development.

Conclusions

The use of silk fibroin (SF) as a drug delivery system combines remarkable features, particularly in light of musculoskeletal application. These include the (i) the stabilization of drugs entrapped in SF, (ii) option to tailor release kinetics as a function of SF crystallinity and degradation mechanism, (iii) relatively easy manufacturing in all aqueous processes, (iv) easy sterilization possibilities and (v) advanced mechanical properties. In addition, the natural gelification process has recently been mimicked to an extent, so that instantaneous solidification or gelification may become feasible, arguably one of the requirements to overcome serious limitations of current injectable drug delivery systems and allowing intraarticular use of SF. Exciting new delivery platforms have been presented recently, including drug delivery from microneedles, presentation of SF-protein drug bioconjugates, or functionalized SF scaffolds with tailored surface charge to fine-tune protein drug delivery in a biomimetic fashion. These promising advances and demonstrated potential at the drug delivery / biomaterial interface, is expected to lead into future studies within which results from relevant animal model systems will support the move from the lab bench to clinical application in a truly translational fashion.

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References

1. Lubec G, Halaubek J, Feldl C, Lubec B, Strouhal E. Use of Silk in Ancient-Egypt. *Nature*. 1993; 362:25–25.
2. Uebersax L, Merkle HP, Meinel L. Biopolymer-based growth factor delivery for tissue repair: from natural concepts to engineered systems. *Tissue Eng Part B Rev*. 2009; 15:263–289. [PubMed: 19416020]
3. Huemmerich D, Scheibel T, Vollrath F, Cohen S, Gat U, Ittah S. Novel assembly properties of recombinant spider dragline silk proteins. *Curr Biol*. 2004; 14:2070–2074. [PubMed: 15556872]
4. Knight DP, Vollrath F. Changes in element composition along the spinning duct in a *Nephila* spider. *Naturwissenschaften*. 2001; 88:179–182. [PubMed: 11480706]
5. Kaplan, DL.; Mello, CM.; Arcidiacono, S.; Fossey, SA.; Senecal, K.; Muller, W. Silk. In: McGrath, KP.; Kaplan, DL., editors. *Protein-based materials*. Birkhauser; Boston: 1997. p. 103-131.

6. Rockwood DN, Preda RC, Yucel T, Wang X, Lovett ML, Kaplan DL. Materials fabrication from *Bombyx mori* silk fibroin. *Nat Protoc.* 2011; 6:1612–1631. [PubMed: 21959241]
7. Hofmann, S.; Kaplan, DL.; Vunjak-Novakovic, G.; Meinel, L. Tissue engineering of bone. In: Vunjak-Novakovic, G.; Freshney, RI., editors. *Culture of cells for tissue engineering.* John Wiley & Sons, Inc.; Hoboken, New Jersey: 2006. p. 323-375.
8. Acharya C, Ghosh SK, Kundu SC. Silk fibroin protein from mulberry and non-mulberry silkworms: cytotoxicity, biocompatibility and kinetics of L929 murine fibroblast adhesion. *J Mater Sci-Mater M.* 2008; 19:2827–2836. [PubMed: 18322779]
9. Trefiletti V, Conio G, Pioli F, Cavazza B, Perico A, Patrone E. The Spinning of Silk .1. Molecular-Weight, Subunit Structure, and Molecular Shape of *Bombyx-Mori* Fibroin. *Makromol Chem.* 1980; 181:1159–1179.
10. Omenetto FG, Kaplan DL. New opportunities for an ancient material. *Science.* 2010; 329:528–531. [PubMed: 20671180]
11. Phillips DM, Drummy LF, Conrady DG, Fox DM, Naik RR, Stone MO, Trulove PC, De Long HC, Mantz RA. Dissolution and regeneration of *Bombyx mori* silk fibroin using ionic liquids. *J Am Chem Soc.* 2004; 126:14350–14351. [PubMed: 15521743]
12. Murphy AR, St John P, Kaplan DL. Modification of silk fibroin using diazonium coupling chemistry and the effects on hMSC proliferation and differentiation. *Biomaterials.* 2008; 29:2829–2838. [PubMed: 18417206]
13. Nakamura, SM.; Magoshi, JY. Silk polymers: Material Science and Biotechnology. In: Kaplan, DL.; Adams, WW.; Farmer, B.; Viney, C., editors. *ACS Symp Ser.* 1994. p. 211-221.
14. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL. Silk-based biomaterials. *Biomaterials.* 2003; 24:401–416. [PubMed: 12423595]
15. Meinel L, Hofmann S, Karageorgiou V, Kirker-Head C, McCool J, Gronowicz G, Zichner L, Langer R, Vunjak-Novakovic G, Kaplan DL. The inflammatory responses to silk films in vitro and in vivo. *Biomaterials.* 2005; 26:147–155. [PubMed: 15207461]
16. Wang Y, Rudym DD, Walsh A, Abrahamsen L, Kim HJ, Kim HS, Kirker-Head C, Kaplan DL. In vivo degradation of three-dimensional silk fibroin scaffolds. *Biomaterials.* 2008; 29:3415–3428. [PubMed: 18502501]
17. Whyte, MP.; Greenberg, CR.; Edgar, T.; Van Sickle, BJ.; Hamdan, M.; Salman, N.; Bober, M.; McAlister, WH.; Wenkert, D.; Landy, H. Hypophosphatasia: Treatment of life-threatening disease using bone-targeted human recombinant tissue non-specific alkaline phosphatase. 59th Annual Meeting of the American Society of Human Genetics; Honolulu. 2009. abstract; program #138
18. Wenkert D, McAlister WH, Coburn SP, Zerega JA, Ryan LM, Ericson KL, Hersch JH, Mumm S, Whyte MP. Hypophosphatasia: nonlethal disease despite skeletal presentation in utero (17 new cases and literature review). *J Bone Miner Res.* 2011; 26:2389–2398. [PubMed: 21713987]
19. Hermeling S, Crommelin DJ, Schellekens H, Jiskoot W. Structure-immunogenicity relationships of therapeutic proteins. *Pharm Res.* 2004; 21:897–903. [PubMed: 15212151]
20. Zhang YQ, Ma Y, Xia YY, Shen WD, Mao JP, Zha XM, Shirai K, Kiguchi K. Synthesis of silk fibroin-insulin bioconjugates and their characterization and activities in vivo. *J Biomed Mater Res B Appl Biomater.* 2006; 79:275–283. [PubMed: 16767720]
21. Keating MJ, Holmes R, Lerner S, Ho DH. L-asparaginase and PEG asparaginase--past, present, and future. *Leuk Lymphoma.* 1993; 10(Suppl):153–157. [PubMed: 8481665]
22. Zhang YQ, Zhou WL, Shen WD, Chen YH, Zha XM, Shirai K, Kiguchi K. Synthesis, characterization and immunogenicity of silk fibroin-L-asparaginase bioconjugates. *J Biotechnol.* 2005; 120:315–326. [PubMed: 16102867]
23. Schellekens H. How to predict and prevent the immunogenicity of therapeutic proteins. *Biotechnol Annu Rev.* 2008; 14:191–202. [PubMed: 18606364]
24. Barak V, Acker M, Nisman B, Kalickman I, Abrahamov A, Zimran A, Yatziv S. Cytokines in Gaucher's disease. *Eur Cytokine Netw.* 1999; 10:205–210. [PubMed: 10400826]
25. Wenk E, Merkle HP, Meinel L. Silk fibroin as a vehicle for drug delivery applications. *J Control Release.* 2010

26. Decuzzi P, Godin B, Tanaka T, Lee SY, Chiappini C, Liu X, Ferrari M. Size and shape effects in the biodistribution of intravascularly injected particles. *J Control Release*. 2010; 141:320–327. [PubMed: 19874859]
27. Melton LJ 3rd, Thamer M, Ray NF, Chan JK, Chesnut CH 3rd, Einhorn TA, Johnston CC, Raisz LG, Silverman SL, Siris ES. Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation. *J Bone Miner Res*. 1997; 12:16–23. [PubMed: 9240721]
28. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res*. 2000; 15:2–12. [PubMed: 10646108]
29. Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley ER, Kurokawa T, Suda T. Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. *J Clin Invest*. 1993; 91:257–263. [PubMed: 8423223]
30. Baron R, Hesse E. Update on bone anabolics in osteoporosis treatment: rationale, current status, and perspectives. *J Clin Endocrinol Metab*. 2012; 97:311–325. [PubMed: 22238383]
31. Ominsky MS, Vlasseros F, Jolette J, Smith SY, Stouch B, Doellgast G, Gong J, Gao Y, Cao J, Graham K, Tipton B, Cai J, Deshpande R, Zhou L, Hale MD, Lightwood DJ, Henry AJ, Popplewell AG, Moore AR, Robinson MK, Lacey DL, Simonet WS, Paszty C. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J Bone Miner Res*. 2010; 25:948–959. [PubMed: 20200929]
32. Lammel AS, Hu X, Park SH, Kaplan DL, Scheibel TR. Controlling silk fibroin particle features for drug delivery. *Biomaterials*. 2010; 31:4583–4591. [PubMed: 20219241]
33. Mathur AB, Gupta V. Silk fibroin-derived nanoparticles for biomedical applications. *Nanomedicine (Lond)*. 2010; 5:807–820. [PubMed: 20662650]
34. Wang X, Yucel T, Lu Q, Hu X, Kaplan DL. Silk nanospheres and microspheres from silk/pva blend films for drug delivery. *Biomaterials*. 2010; 31:1025–1035. [PubMed: 19945157]
35. 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. [PubMed: 18761384]
36. Park JH, Allen MG, Prausnitz MR. Polymer microneedles for controlled-release drug delivery. *Pharm Res*. 2006; 23:1008–1019. [PubMed: 16715391]
37. Kim YC, Quan FS, Compans RW, Kang SM, Prausnitz MR. Stability kinetics of influenza vaccine coated onto microneedles during drying and storage. *Pharm Res*. 2011; 28:135–144. [PubMed: 20387097]
38. Tsiolis K, Waseem RR, Pritchard EM, Panilaitis B, Kaplan DL, Omenetto F. Fabrication of silk microneedles for controlled-release drug delivery. *Adv Funct Mater*. 2012; 22:350–355.
39. Arora A, Prausnitz MR, Mitragotri S. Micro-scale devices for transdermal drug delivery. *Int J Pharm*. 2008; 364:227–236. [PubMed: 18805472]
40. Kirker-Head C, Karageorgiou V, Hofmann S, Fajardo R, Betz O, Merkle HP, Hilbe M, von Rechenberg B, McCool J, Abrahamsen L, Nazarian A, Cory E, Curtis M, Kaplan D, Meinel L. BMP-silk composite matrices heal critically sized femoral defects. *Bone*. 2007; 41:247–255. [PubMed: 17553763]
41. Kirker-Head C, Karageorgiou V, Hofmann S, Fajardo R, Betz O, Merkle HP, Hilbe M, von Rechenberg B, McCool J, Abrahamsen L, Nazarian A, Cory E, Curtis M, Kaplan D, Meinel L. BMP-silk composite matrices heal critically sized femoral defects. *Bone*. 2007; 41:247. 43 (2008) 1124–1124. [PubMed: 17553763]
42. Min S, Gao X, Han C, Chen Y, Yang M, Zhu L, Zhang H, Liu L, Yao J. Preparation of a silk fibroin spongy wound dressing and its therapeutic efficiency in skin defects. *J Biomater Sci Polym Ed*. 2012; 23:97–110. [PubMed: 21176393]
43. Hofmann S, Foo CT, Rossetti F, Textor M, Vunjak-Novakovic G, Kaplan DL, Merkle HP, Meinel L. Silk fibroin as an organic polymer for controlled drug delivery. *J Control Release*. 2006; 111:219–227. [PubMed: 16458987]
44. 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. [PubMed: 18280603]

45. Wenk E, Merkle HP, Meinel L. Silk fibroin as a vehicle for drug delivery applications. *J Control Release*. 2011; 150:128–141. [PubMed: 21059377]
46. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-1 directly regulate bone growth and density. *J Clin Invest*. 2002; 110:771–781. [PubMed: 12235108]
47. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyeva Y, Kline WO, Gonzalez M, Yancopoulos GD, Glass DJ. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell*. 2004; 14:395–403. [PubMed: 15125842]
48. Lane, NE.; Cosman, F.; Bolognese, MA.; Zanchetta, J.; Sees, K.; Gaumer, K.; Daddona, EE. Transdermal delivery of (hPTH 1-34) (ZP-PTH) is effective in increasing bone mineral density of the lumbar spine & hip in postmenopausal women with osteoporosis. ACR/ARHP; San Francisco, CA: 2008.
49. Cosman F, Lane NE, Bolognese MA, Zanchetta JR, Garcia-Hernandez PA, Sees K, Matriano JA, Gaumer K, Daddona PE. Effect of transdermal teriparatide administration on bone mineral density in postmenopausal women. *J Clin Endocrinol Metab*. 2010; 95:151–158. [PubMed: 19858319]
50. Ameri M, Fan SC, Maa YF. Parathyroid hormone PTH(1-34) formulation that enables uniform coating on a novel transdermal microprojection delivery system. *Pharm Res*. 2010; 27:303–313. [PubMed: 20013035]
51. Pritchard EM, Dennis PB, Omenetto F, Naik RR, Kaplan DL. Physical and chemical aspects of stabilization of compounds in silk. *Biopolymers*. 2012
52. Wenk E, Murphy AR, Kaplan DL, Meinel L, Merkle HP, Uebersax L. The use of sulfonated silk fibroin derivatives to control binding, delivery and potency of FGF-2 in tissue regeneration. *Biomaterials*. 2010; 31:1403–1413. [PubMed: 19942287]
53. Choi HM, Bide M, Phaneuf M, Quist W, LoGerfo F. Dyeing of wool with antibiotics to develop novel infection resistance materials for extracorporeal end use. *J Appl Polym Sci*. 2004; 92:3343–3354.
54. Choi HM, Bide M, Phaneuf M, Quist W, Logerfo F. Antibiotic treatment of silk to produce novel infection-resistant biomaterials. *Text Res J*. 2004; 74:333–342.
55. Brisaert M, Heylen M, Plaizier-Vercammen J. Investigation on the chemical stability of erythromycin in solutions using an optimization system. *Pharm World Sci*. 1996; 18:182–186. [PubMed: 8933579]
56. Zhang X, Reagan MR, Kaplan DL. Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev*. 2009; 61:988–1006. [PubMed: 19643154]
57. Kluge JA, Rabotyagova O, Leisk GG, Kaplan DL. Spider silks and their applications. *Trends Biotechnol*. 2008; 26:244–251. [PubMed: 18367277]
58. Gupta V, Mun GH, Choi B, Aseh A, Mildred L, Patel A, Zhang Q, Price JE, Chang D, Robb G, Mathur AB. Repair and reconstruction of a resected tumor defect using a composite of tissue flap-nanotherapeutic-silk fibroin and chitosan scaffold. *Ann Biomed Eng*. 2011; 39:2374–2387. [PubMed: 21706247]
59. Rios CN, Skoracki RJ, Miller MJ, Satterfield WC, Mathur AB. In vivo bone formation in silk fibroin and chitosan blend scaffolds via ectopically grafted periosteum as a cell source: a pilot study. *Tissue Eng Part A*. 2009; 15:2717–2725. [PubMed: 19718840]
60. Meinel L, Fajardo R, Hofmann S, Langer R, Chen J, Snyder B, Vunjak-Novakovic G, Kaplan D. Silk implants for the healing of critical size bone defects. *Bone*. 2005; 37:688–698. [PubMed: 16140599]
61. Meinel L, Fajardo R, Hofmann S, Langer R, Chen J, Snyder B, Vunjak-Novakovic G, Kaplan D. Silk implants for the healing of critical size bone defects. *Bone*. 2005; 37:688. 43 (2008) 1122-1122. [PubMed: 16140599]
62. Hakimi O, Vollrath F, Carr A. Evaluation of Silk as a Scaffold for Musculoskeletal Regeneration – the Path from the Laboratory to Clinical Trials. *Comprehensive Biotechnology*. 2011; 5:341–351.
63. Meinel L, Hofmann S, Betz O, Fajardo R, Merkle HP, Langer R, Evans CH, Vunjak-Novakovic G, Kaplan DL. Osteogenesis by human mesenchymal stem cells cultured on silk biomaterials:

- comparison of adenovirus mediated gene transfer and protein delivery of BMP-2. *Biomaterials*. 2006; 27:4993–5002. [PubMed: 16765437]
64. West RB, Rubin BP, Miller MA, Subramanian S, Kaygusuz G, Montgomery K, Zhu S, Marinelli RJ, De Luca A, Downs-Kelly E, Goldblum JR, Corless CL, Brown PO, Gilks CB, Nielsen TO, Huntsman D, van de Rijn M. A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. *Proc Natl Acad Sci U S A*. 2006; 103:690–695. [PubMed: 16407111]
 65. Cupp JS, Miller MA, Montgomery KD, Nielsen TO, O'Connell JX, Huntsman D, van de Rijn M, Gilks CB, West RB. Translocation and expression of CSF1 in pigmented villonodular synovitis, tenosynovial giant cell tumor, rheumatoid arthritis and other reactive synovitides. *Am J Surg Pathol*. 2007; 31:970–976. [PubMed: 17527089]
 66. Blay JY, El Sayadi H, Thiesse P, Garret J, Ray-Coquard I. Complete response to imatinib in relapsing pigmented villonodular synovitis/tenosynovial giant cell tumor (PVNS/TGCT). *Ann Oncol*. 2008; 19:821–822. [PubMed: 18296418]
 67. Elia R, Newhide DR, Pedevillano PD, Reiss GR, Firpo MA, Hsu EW, Kaplan DL, Prestwich GD, Peattie RA. Silk-hyaluronan-based composite hydrogels: A novel, securable vehicle for drug delivery. *J Biomater Appl*. 2011
 68. Fini M, Motta A, Torricelli P, Giavaresi G, Nicoli Aldini N, Tschon M, Giardino R, Migliaresi C. The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel. *Biomaterials*. 2005; 26:3527–3536. [PubMed: 15621243]
 69. Kaplan DL, Kim UJ, Park JY, Li CM, Jin HJ, Valluzzi R. Structure and properties of silk hydrogels. *Biomacromolecules*. 2004; 5:786–792. [PubMed: 15132662]
 70. Kim UJ, Park J, Li C, Jin HJ, Valluzzi R, Kaplan DL. Structure and properties of silk hydrogels. *Biomacromolecules*. 2004; 5:786–792. [PubMed: 15132662]
 71. Kluge JA, Rosiello NC, Leisk GG, Kaplan DL, Dorfmann AL. The consolidation behavior of silk hydrogels. *J Mech Behav Biomed Mater*. 2010; 3:278–289. [PubMed: 20142112]
 72. Zhang W, Wang X, Wang S, Zhao J, Xu L, Zhu C, Zeng D, Chen J, Zhang Z, Kaplan DL, Jiang X. The use of injectable sonication-induced silk hydrogel for VEGF(165) and BMP-2 delivery for elevation of the maxillary sinus floor. *Biomaterials*. 2011
 73. Kim HJ, Kim UJ, Kim HS, Li C, Wada M, Leisk GG, Kaplan DL. Bone tissue engineering with premineralized silk scaffolds. *Bone*. 2008; 42:1226–1234. [PubMed: 18387349]
 74. Garcia-Fuentes M, Giger E, Meinel L, Merkle HP. The effect of hyaluronic acid on silk fibroin conformation. *Biomaterials*. 2008; 29:633–642. [PubMed: 17996295]
 75. Jin HJ, Park J, Karageorgiou V, Kim UJ, Valluzzi R, Kaplan DL. Water-stable silk films with reduced beta-sheet content. *Adv Funct Mater*. 2005; 15:1241–1247.
 76. Li CM, Jin HJ, Botsaris GD, Kaplan DL. Functionalized electrospun silk fibers as template for biomineralization. *Abstr Pap Am Chem S*. 2003; 226:U498–U499.
 77. Freddi G, Arai T, Innocenti R, Tsukada M. Biodegradation of Bombyx mori silk fibroin fibers and films. *J Appl Polym Sci*. 2004; 91:2383–2390.
 78. Park SH, Gil ES, Kim HJ, Lee K, Kaplan DL. Relationships between degradability of silk scaffolds and osteogenesis. *Biomaterials*. 2010; 31:6162–6172. [PubMed: 20546890]
 79. Jin HJ, Kaplan DL. Mechanism of silk processing in insects and spiders. *Nature*. 2003; 424:1057–1061. [PubMed: 12944968]
 80. Valluzzi R, Winkler S, Wilson D, Kaplan DL. Silk: molecular organization and control of assembly. *Philos Trans R Soc Lond B Biol Sci*. 2002; 357:165–167. [PubMed: 11911773]
 81. Kaplan DL. Spiderless spider webs. *Nat Biotechnol*. 2002; 20:239–240. [PubMed: 11875422]
 82. Rabotyagova OS, Cebe P, Kaplan DL. Role of polyalanine domains in beta-sheet formation in spider silk block copolymers. *Macromol Biosci*. 2010; 10:49–59. [PubMed: 19890885]
 83. Kaplan DL, Wang XQ, Wenk E, Matsumoto A, Meinel L, Li CM. Silk microspheres for encapsulation and controlled release. *Journal of Controlled Release*. 2007; 117:360–370. [PubMed: 17218036]
 84. Numata K, Kaplan DL. Silk-based delivery systems of bioactive molecules. *Adv Drug Deliv Rev*. 2010; 62:1497–1508. [PubMed: 20298729]

85. Lu Q, Hu X, Wang X, Kluge JA, Lu S, Cebe P, Kaplan DL. Water-insoluble silk films with silk I structure. *Acta Biomater.* 2010; 6:1380–1387. [PubMed: 19874919]
86. Numata K, Cebe P, Kaplan DL. Mechanism of enzymatic degradation of beta-sheet crystals. *Biomaterials.* 2010; 31:2926–2933. [PubMed: 20044136]
87. Lu Q, Zhang B, Li M, Zuo B, Kaplan DL, Huang Y, Zhu H. Degradation mechanism and control of silk fibroin. *Biomacromolecules.* 2011; 12:1080–1086. [PubMed: 21361368]
88. Minoura N, Li MZ, Ogiso M. Enzymatic degradation behavior of porous silk fibroin sheets. *Biomaterials.* 2003; 24:357–365. [PubMed: 12419638]
89. Horan RL, Antle K, Collette AL, Wang Y, Huang J, Moreau JE, Volloch V, Kaplan DL, Altman GH. In vitro degradation of silk fibroin. *Biomaterials.* 2005; 26:3385–3393. [PubMed: 15621227]
90. Panilaitis B, Altman GH, Chen J, Jin HJ, Karageorgiou V, Kaplan DL. Macrophage responses to silk. *Biomaterials.* 2003; 24:3079–3085. [PubMed: 12895580]
91. Meinel L, Betz O, Fajardo R, Hofmann S, Nazarian A, Cory E, Hilbe M, McCool J, Langer R, Vunjak-Novakovic G, Merkle HP, Rechenberg B, Kaplan DL, Kirker-Head C. Silk based biomaterials to heal critical sized femur defects. *Bone.* 2006; 39:922–931. [PubMed: 16757219]
92. Wenk E, Meinel AJ, Wildy S, Merkle HP, Meinel L. Microporous silk fibroin scaffolds embedding PLGA microparticles for controlled growth factor delivery in tissue engineering. *Biomaterials.* 2009; 30:2571–2581. [PubMed: 19157533]
93. Hagenmuller H, Merkle HP, Meinel L, Muller R. Combining time-lapsed mu CT monitoring and mechanical stimulation in bone tissue engineering. *Tissue Engineering.* 2007; 13:1660–1660.
94. Uebersax L, Hagenmuller H, Hofmann S, Gruenblatt E, Muller R, Vunjak-Novakovic G, Kaplan DL, Merkle HP, Meinel L. Effect of scaffold design on bone morphology in vitro. *Tissue Eng.* 2006; 12:3417–3429. [PubMed: 17518678]
95. Meinel L, Karageorgiou V, Fajardo R, Snyder B, Shinde-Patil V, Zichner L, Kaplan D, Langer R, Vunjak-Novakovic G. Bone tissue engineering using human mesenchymal stem cells: effects of scaffold material and medium flow. *Ann Biomed Eng.* 2004; 32:112–122. [PubMed: 14964727]
96. Blackburn J, Hodgkinson R, Currey JD, Mason JE. Mechanical properties of microcallus in human cancellous bone. *J Orthop Res.* 1992; 10:237–246. [PubMed: 1740742]
97. Cullinane, DM.; Einhorn, TA. Biomechanics of bone. In: Bilezikian, JP.; Raisz, LG.; Rodan, GA., editors. *Principles in bone biology.* Academic Press; San Diego: 2001. p. 17-32.
98. Zhao J, Zhang Z, Wang S, Sun X, Zhang X, Chen J, Kaplan DL, Jiang X. Apatite-coated silk fibroin scaffolds to healing mandibular border defects in canines. *Bone.* 2009; 45:517–527. [PubMed: 19505603]
99. Gil ES, Kluge JA, Rockwood DN, Rajkhowa R, Wang L, Wang X, Kaplan DL. Mechanical improvements to reinforced porous silk scaffolds. *J Biomed Mater Res A.* 2011; 99:16–28. [PubMed: 21793193]
100. Rajkhowa R, Gil ES, Kluge J, Numata K, Wang L, Wang X, Kaplan DL. Reinforcing silk scaffolds with silk particles. *Macromol Biosci.* 2010; 10:599–611. [PubMed: 20166230]
101. Cunniff PM, Fossey SA, Auerbach MA, Song JW. Mechanical-Properties of Major Ampulate Gland Silk Fibers Extracted from Nephila-Clavipes Spiders. *Acs Sym Ser.* 1994; 544:234–251.
102. Halsted W. The employment of fine silk in preference to catgut and the advantage of transfixing tissues and vessels in controlling hemorrhage. *Ann Surg.* 1892; 16:505.
103. Lange F. Über die Sehnenplastik. *Verh Dtsch Orthop Ges.* 1903; 2:10–12.
104. Lange F. Künstliche Bänder aus Seide. *Münch Med Wochenschr.* 1907; 17:834–836.
105. Serica, SeriACL™ Device Trial for Anterior Cruciate Ligament (ACL) Reconstruction. 2008. www.ClinTrials.gov
106. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, Kaplan DL. Silk matrix for tissue engineered anterior cruciate ligaments. *Biomaterials.* 2002; 23:4131–4141. [PubMed: 12182315]
107. Meinel L, Hoffman S, Betz O, Fajardo R, Kaplan D. Human mesenchymal stem cell differentiation in response to gene and protein BMP2 delivery. *Abstr Pap Am Chem S.* 2004; 227:U201–U201.

108. Meinel L, Karageorgiou V, Hofmann S, Fajardo R, Snyder B, Li C, Zichner L, Langer R, Vunjak-Novakovic G, Kaplan DL. Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. *J Biomed Mater Res A*. 2004; 71:25–34. [PubMed: 15316936]
109. Boison D, Uebersax L, Fedele DE, Schumacher C, Kaplan DL, Merkle HP, Meinel L. The support of adenosine release from adenosine kinase deficient ES cells by silk substrates. *Biomaterials*. 2006; 27:4599–4607. [PubMed: 16709437]
110. Hutchinson GE. Thoughts on Aquatic Insects. *Bioscience*. 1981; 31:495–500.
111. Malas D, Wallace JB. Strategies for Coexistence in 3 Species of Net-Spinning Caddisflies (Trichoptera) in 2nd-Order Southern Appalachian Streams. *Can J Zool*. 1977; 55:1829–1840.
112. Sahoo S, Toh SL, Goh JC. A bFGF-releasing silk/PLGA-based biohybrid scaffold for ligament/tendon tissue engineering using mesenchymal progenitor cells. *Biomaterials*. 2010; 31:2990–2998. [PubMed: 20089300]
113. Jin HJ, Park J, Valluzzi R, Cebe P, Kaplan DL. Biomaterial films of Bombyx mori silk fibroin with poly(ethylene oxide). *Biomacromolecules*. 2004; 5:711–717. [PubMed: 15132651]
114. Lu S, Wang X, Lu Q, Zhang X, Kluge JA, Uppal N, Omenetto F, Kaplan DL. Insoluble and flexible silk films containing glycerol. *Biomacromolecules*. 2010; 11:143–150. [PubMed: 19919091]
115. Garcia-Fuentes M, Meinel AJ, Hilbe M, Meinel L, Merkle HP. Silk fibroin/hyaluronan scaffolds for human mesenchymal stem cell culture in tissue engineering. *Biomaterials*. 2009; 30:5068–5076. [PubMed: 19564040]
116. Arakawa T, Prestrelski SJ, Kenney WC, Carpenter JF. Factors affecting short-term and long-term stabilities of proteins. *Adv Drug Deliver Rev*. 2001; 46:307–326.
117. Arakawa T, Kita Y, Carpenter JF. Protein-Solvent Interactions in Pharmaceutical Formulations. *Pharmaceut Res*. 1991; 8:285–291.
118. Augst A, Marolt D, Freed LE, Vepari C, Meinel L, Farley M, Fajardo R, Patel N, Gray M, Kaplan DL, Vunjak-Novakovic G. Effects of chondrogenic and osteogenic regulatory factors on composite constructs grown using human mesenchymal stem cells, silk scaffolds and bioreactors. *J R Soc Interface*. 2008; 5:929–939. [PubMed: 18230586]
119. Karageorgiou V, Meinel L, Hofmann S, Malhotra A, Volloch V, Kaplan D. Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells. *J Biomed Mater Res A*. 2004; 71:528–537. [PubMed: 15478212]
120. Uebersax L, Mattotti M, Papaloizos M, Merkle HP, Gander B, Meinel L. Silk fibroin matrices for the controlled release of nerve growth factor (NGF). *Biomaterials*. 2007; 28:4449–4460. [PubMed: 17643485]
121. Murphy AR, Kaplan DL. Biomedical applications of chemically-modified silk fibroin. *J Mater Chem*. 2009; 19:6443–6450. [PubMed: 20161439]
122. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol*. 1988; 15:1833–1840. [PubMed: 3068365]
123. Felson DT, Lawrence RC, Hochberg MC, McAlindon T, Dieppe PA, Minor MA, Blair SN, Berman BM, Fries JF, Weinberger M, Lorig KR, Jacobs JJ, Goldberg V. Osteoarthritis: new insights. Part 2: treatment approaches. *Ann Intern Med*. 2000; 133:726–737. [PubMed: 11074906]
124. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y, Sowers M, McAlindon T, Spector TD, Poole AR, Yanovski SZ, Ateshian G, Sharma L, Buckwalter JA, Brandt KD, Fries JF. Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Ann Intern Med*. 2000; 133:635–646. [PubMed: 11033593]
125. Szerb I, Hangody L, Duska Z, Kaposi NP. Mosaicplasty: long-term follow-up. *Bull Hosp Jt Dis*. 2005; 63:54–62. [PubMed: 16536220]
126. Reddy S, Pedowitz DI, Parekh SG, Sennett BJ, Okereke E. The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. *Am J Sports Med*. 2007; 35:80–85. [PubMed: 16957009]

127. Mano JF, Reis RL. Osteochondral defects: present situation and tissue engineering approaches. *J Tissue Eng Regen Med*. 2007; 1:261–273. [PubMed: 18038416]
128. Heywood HK, Bader DL, Lee DA. Glucose concentration and medium volume influence cell viability and glycosaminoglycan synthesis in chondrocyte-seeded alginate constructs. *Tissue Eng*. 2006; 12:3487–3496. [PubMed: 17518685]
129. Meinel, AJ. Chemistry and Pharmaceutical Sciences. ETH Zurich; Zurich: 2010. Cell Instructive Silk Fibroin Scaffolds for Tissue Engineering.
130. Wang X, Wenk E, Matsumoto A, Meinel L, Li C, Kaplan DL. Silk microspheres for encapsulation and controlled release. *J Control Release*. 2007; 117:360–370. [PubMed: 17218036]
131. Lammel A, Schwab M, Hofer M, Winter G, Scheibel T. Recombinant spider silk particles as drug delivery vehicles. *Biomaterials*. 2011; 32:2233–2240. [PubMed: 21186052]
132. Hofer M, Winter G, Myschik J. Recombinant spider silk particles for controlled delivery of protein drugs. *Biomaterials*. 2012; 33:1554–1562. [PubMed: 22079006]
133. Meinel L, Zoidis E, Zapf J, Hassa P, Hottiger MO, Auer JA, Schneider R, Gander B, Luginbuehl V, Bettschart-Wolfisberger R, Illi OE, Merkle HP, von Rechenberg B. Localized insulin-like growth factor I delivery to enhance new bone formation. *Bone*. 2003; 33:660–672. [PubMed: 14555272]
134. Meinel L, Illi OE, Zapf J, Malfanti M, Peter Merkle H, Gander B. Stabilizing insulin-like growth factor-I in poly(D,L-lactide-co-glycolide) microspheres. *J Control Release*. 2001; 70:193–202. [PubMed: 11166419]
135. Luginbuehl V, Meinel L, Merkle HP, Gander B. Localized delivery of growth factors for bone repair. *Eur J Pharm Biopharm*. 2004; 58:197–208. [PubMed: 15296949]
136. Luginbuehl V, Wenk E, Koch A, Gander B, Merkle HP, Meinel L. Insulin-like growth factor I-releasing alginate-tricalciumphosphate composites for bone regeneration. *Pharm Res*. 2005; 22:940–950. [PubMed: 15948038]
137. Gobin AS, Rhea R, Newman RA, Mathur AB. Silk-fibroin-coated liposomes for long-term and targeted drug delivery. *Int J Nanomedicine*. 2006; 1:81–87. [PubMed: 17722265]
138. Gotoh K, Izumi H, Kanamoto T, Tamada Y, Nakashima H. Sulfated fibroin, a novel sulfated peptide derived from silk, inhibits human immunodeficiency virus replication in vitro. *Biosci Biotechnol Biochem*. 2000; 64:1664–1670. [PubMed: 10993153]
139. Tamada Y. Sulfation of silk fibroin by chlorosulfonic acid and the anticoagulant activity. *Biomaterials*. 2004; 25:377–383. [PubMed: 14585685]
140. Taddei P, Arosio C, Monti P, Tsukada M, Arai T, Freddi G. Chemical and physical properties of sulfated silk fabrics. *Biomacromolecules*. 2007; 8:1200–1208. [PubMed: 17338562]
141. Kaplan DL, Meinel L, Hofmann S, Karageorgiou V, Kirker-Head C, McCool J, Gronowicz G, Zichner L, Langer R, Vunjak-Novakovic G. The inflammatory responses to silk films in vitro and in vivo. *Biomaterials*. 2005; 26:147–155. [PubMed: 15207461]
142. Hofmann S, Knecht S, Langer R, Kaplan DL, Vunjak-Novakovic G, Merkle HP, Meinel L. Cartilage-like tissue engineering using silk scaffolds and mesenchymal stem cells. *Tissue Eng*. 2006; 12:2729–2738. [PubMed: 17518642]
143. Gotoh Y, Niimi S, Hayakawa T, Miyashita T. Preparation of lactose-silk fibroin conjugates and their application as a scaffold for hepatocyte attachment. *Biomaterials*. 2004; 25:1131–1140. [PubMed: 14615179]
144. Acharya C, Hinz B, Kundu SC. The effect of lactose-conjugated silk biomaterials on the development of fibrogenic fibroblasts. *Biomaterials*. 2008; 29:4665–4675. [PubMed: 18804860]
145. Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem*. 1999; 68:729–777. [PubMed: 10872465]
146. Takada T, Katagiri T, Ifuku M, Morimura N, Kobayashi M, Hasegawa K, Ogamo A, Kamijo R. Sulfated polysaccharides enhance the biological activities of bone morphogenetic proteins. *J Biol Chem*. 2003; 278:43229–43235. [PubMed: 12912996]

147. Molteni A, Modrowski D, Hott M, Marie PJ. Alterations of matrix- and cell-associated proteoglycans inhibit osteogenesis and growth response to fibroblast growth factor-2 in cultured rat mandibular condyle and calvaria. *Cell Tissue Res.* 1999; 295:523–536. [PubMed: 10022972]
148. Lu Q, Zhang S, Hu K, Feng Q, Cao C, Cui F. Cytocompatibility and blood compatibility of multifunctional fibroin/collagen/heparin scaffolds. *Biomaterials.* 2007; 28:2306–2313. [PubMed: 17292467]
149. Bhardwaj N, Nguyen QT, Chen AC, Kaplan DL, Sah RL, Kundu SC. Potential of 3-D tissue constructs engineered from bovine chondrocytes/silk fibroin-chitosan for in vitro cartilage tissue engineering. *Biomaterials.* 2011; 32:5773–5781. [PubMed: 21601277]
150. Hatefi A, Amsden B. Biodegradable injectable in situ forming drug delivery systems. *J Control Release.* 2002; 80:9–28. [PubMed: 11943384]
151. Einmahl S, Behar-Cohen F, Tabatabay C, Savoldelli M, D’Hermies F, Chauvaud D, Heller J, Gurny R. A viscous bioerodible poly(ortho ester) as a new biomaterial for intraocular application. *J Biomed Mater Res.* 2000; 50:566–573. [PubMed: 10756315]
152. Jackson JK, Min W, Cruz TF, Cindric S, Arsenault L, Von Hoff DD, Degan D, Hunter WL, Burt HM. A polymer-based drug delivery system for the antineoplastic agent bis(maltolato)oxovanadium in mice. *Br J Cancer.* 1997; 75:1014–1020. [PubMed: 9083337]
153. Deshpande AA, Heller J, Gurny R. Bioerodible polymers for ocular drug delivery. *Crit Rev Ther Drug Carrier Syst.* 1998; 15:381–420. [PubMed: 9736417]
154. Walter KA, Cahan MA, Gur A, Tyler B, Hilton J, Colvin OM, Burger PC, Domb A, Brem H. Interstitial taxol delivered from a biodegradable polymer implant against experimental malignant glioma. *Cancer Res.* 1994; 54:2207–2212. [PubMed: 7909720]
155. Winternitz CI, Jackson JK, Oktaba AM, Burt HM. Development of a polymeric surgical paste formulation for taxol. *Pharm Res.* 1996; 13:368–375. [PubMed: 8692727]
156. Jackson JK, Gleave ME, Yago V, Beraldi E, Hunter WL, Burt HM. The suppression of human prostate tumor growth in mice by the intratumoral injection of a slow-release polymeric paste formulation of paclitaxel. *Cancer Res.* 2000; 60:4146–4151. [PubMed: 10945622]
157. Temenoff JS, Mikos AG. Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials.* 2000; 21:2405–2412. [PubMed: 11055288]
158. He S, Yaszemski MJ, Yasko AW, Engel PS, Mikos AG. Injectable biodegradable polymer composites based on poly(propylene fumarate) crosslinked with poly(ethylene glycol)-dimethacrylate. *Biomaterials.* 2000; 21:2389–2394. [PubMed: 11055286]
159. Suggs LJ, Mikos AG. Development of poly(propylene fumarate-co-ethylene glycol) as an injectable carrier for endothelial cells. *Cell Transplant.* 1999; 8:345–350. [PubMed: 10478714]
160. Lu S, Anseth KS. Photopolymerization of multilaminated poly(HEMA) hydrogels for controlled release. *J Control Release.* 1999; 57:291–300. [PubMed: 9895416]
161. Luan X, Bodmeier R. In situ forming microparticle system for controlled delivery of leuprolide acetate: influence of the formulation and processing parameters. *Eur J Pharm Sci.* 2006; 27:143–149. [PubMed: 16243496]
162. Rungseewijitprapa W, Bodmeier R. Injectability of biodegradable in situ forming microparticle systems (ISM). *Eur J Pharm Sci.* 2009; 36:524–531. [PubMed: 19124076]
163. Jeong B, Bae YH, Kim SW. In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *J Biomed Mater Res.* 2000; 50:171–177. [PubMed: 10679681]
164. Paavola A, Yliruusi J, Rosenberg P. Controlled release and dura mater permeability of lidocaine and ibuprofen from injectable poloxamer-based gels. *J Control Release.* 1998; 52:169–178. [PubMed: 9685947]
165. Kranz H, Brazeau GA, Napaporn J, Martin RL, Millard W, Bodmeier R. Myotoxicity studies of injectable biodegradable in-situ forming drug delivery systems. *Int J Pharm.* 2001; 212:11–18. [PubMed: 11165816]
166. Chandrashekar G, Udupa N. Biodegradable injectable implant systems for long term drug delivery using poly (lactic-co-glycolic) acid copolymers. *J Pharm Pharmacol.* 1996; 48:669–674. [PubMed: 8866326]
167. Eliaz RE, Wallach D, Kost J. Delivery of soluble tumor necrosis factor receptor from in-situ forming PLGA implants: in-vivo. *Pharm Res.* 2000; 17:1546–1550. [PubMed: 11303966]

168. Galaev I, Mattiasson B. Thermoreactive water-soluble polymers, nonionic surfactants, and hydrogels as reagents in biotechnology. *Enzyme Microb Technol.* 1993; 15:354–366. [PubMed: 7763627]
169. Ron ES, Bromberg LE. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Adv Drug Deliv Rev.* 1998; 31:197–221. [PubMed: 10837626]
170. Miller DW, Batrakova EV, Waltner TO, Alakhov V, Kabanov AV. Interactions of pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. *Bioconjug Chem.* 1997; 8:649–657. [PubMed: 9327127]
171. Batrakova EV, Han HY, Alakhov V, Miller DW, Kabanov AV. Effects of pluronic block copolymers on drug absorption in Caco-2 cell monolayers. *Pharm Res.* 1998; 15:850–855. [PubMed: 9647349]
172. Petka WA, Harden JL, McGrath KP, Wirtz D, Tirrell DA. Reversible hydrogels from self-assembling artificial proteins. *Science.* 1998; 281:389–392. [PubMed: 9665877]
173. Wang C, Stewart RJ, Kopecek J. Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature.* 1999; 397:417–420. [PubMed: 9989405]
174. Magoshi, J.; Magoshi, Y.; Becker, MA.; Nakamura, M. Biospinning (Silk fiber formation, multiple spinning mechanisms). In: Salamone, JC., editor. *Polymeric materials encyclopedia*. CRC Press; Boca Raton: 1996. p. 9218
175. Knight DP, Vollrath F. Biological liquid crystal elastomers. *Philos Trans R Soc Lond B Biol Sci.* 2002; 357:155–163. [PubMed: 11911772]
176. Vollrath F, Knight DP. Liquid crystalline spinning of spider silk. *Nature.* 2001; 410:541–548. [PubMed: 11279484]
177. Vollrath F, Madsen B, Shao Z. The effect of spinning conditions on the mechanics of a spider's dragline silk. *Proc Biol Sci.* 2001; 268:2339–2346. [PubMed: 11703874]
178. Meinel L, Luginbuehl V, Wenk E, Koch A, Gander B, Merkle HP. Insulin-like growth factor I-releasing alginate-tricalciumphosphate composites for bone regeneration. *Pharmaceut Res.* 2005; 22:940–950.
179. Motta A, Migliaresi C, Faccioni F, Torricelli P, Fini M, Giardino R. Fibroin hydrogels for biomedical applications: preparation, characterization and in vitro cell culture studies. *J Biomater Sci Polym Ed.* 2004; 15:851–864. [PubMed: 15318796]
180. Matsumoto A, Chen J, Collette AL, Kim UJ, Altman GH, Cebe P, Kaplan DL. Mechanisms of silk fibroin sol-gel transitions. *J Phys Chem B.* 2006; 110:21630–21638. [PubMed: 17064118]
181. Gil ES, Spontak RJ, Hudson SM. Effect of beta-sheet crystals on the thermal and rheological behavior of protein-based hydrogels derived from gelatin and silk fibroin. *Macromol Biosci.* 2005; 5:702–709. [PubMed: 16080165]
182. Wang X, Kluge JA, Leisk GG, Kaplan DL. Sonication-induced gelation of silk fibroin for cell encapsulation. *Biomaterials.* 2008; 29:1054–1064. [PubMed: 18031805]
183. Lu Q, Huang Y, Li M, Zuo B, Lu S, Wang J, Zhu H, Kaplan DL. Silk fibroin electrogelation mechanisms. *Acta Biomater.* 2011; 7:2394–2400. [PubMed: 21345387]
184. Yucel T, Cebe P, Kaplan DL. Vortex-induced injectable silk fibroin hydrogels. *Biophys J.* 2009; 97:2044–2050. [PubMed: 19804736]
185. Tai H, Wang W, Vermonden T, Heath F, Hennink WE, Alexander C, Shakesheff KM, Howdle SM. Thermoresponsive and photocrosslinkable PEGMEMA-PPGMA-EGDMA copolymers from a one-step ATRP synthesis. *Biomacromolecules.* 2009; 10:822–828. [PubMed: 19226106]
186. Mayol L, Biondi M, Quaglia F, Fusco S, Borzacchiello A, Ambrosio L, La Rotonda MI. Injectable thermally responsive mucoadhesive gel for sustained protein delivery. *Biomacromolecules.* 2011; 12:28–33. [PubMed: 21142090]
187. Yoshida R, Sakai K, Okano T, Sakurai Y. Drug Release Profiles in the Shrinking Process of Thermoresponsive Poly(N-Isopropylacrylamide-Co-Alkyl Methacrylate) Gels. *Ind Eng Chem Res.* 1992; 31:2339–2345.
188. Elisseeff J, Anseth K, Langer R, Hrkach JS. Synthesis and characterization of photo-cross-linked polymers based on poly(L-lactic acid-co-L-aspartic acid). *Macromolecules.* 1997; 30:2182–2184.
189. Kim BS, Hrkach JS, Langer R. Biodegradable photo-crosslinked poly(ether-ester) networks for lubricious coatings. *Biomaterials.* 2000; 21:259–265. [PubMed: 10646942]

190. Hill-West JL, Chowdhury SM, Slepian MJ, Hubbell JA. Inhibition of thrombosis and intimal thickening by in situ photopolymerization of thin hydrogel barriers. *Proc Natl Acad Sci U S A*. 1994; 91:5967–5971. [PubMed: 8016098]
191. Hill-West JL, Chowdhury SM, Sawhney AS, Pathak CP, Dunn RC, Hubbell JA. Prevention of postoperative adhesions in the rat by in situ photopolymerization of bioresorbable hydrogel barriers. *Obstet Gynecol*. 1994; 83:59–64. [PubMed: 8272310]
192. Sawhney AS, Pathak CP, van Rensburg JJ, Dunn RC, Hubbell JA. Optimization of photopolymerized bioerodible hydrogel properties for adhesion prevention. *J Biomed Mater Res*. 1994; 28:831–838. [PubMed: 8083251]
193. Pathak CP, Sawhney AS, Quinn CP, Hubbell JA. Polyimide-polyethylene glycol block copolymers: synthesis, characterization, and initial evaluation as a biomaterial. *J Biomater Sci Polym Ed*. 1994; 6:313–323. [PubMed: 7841141]
194. Tang Y, Heaysman CL, Willis S, Lewis AL. Physical hydrogels with self-assembled nanostructures as drug delivery systems. *Expert Opin Drug Deliv*. 2011; 8:1141–1159. [PubMed: 21619469]
195. Van Tomme SR, Storm G, Hennink WE. In situ gelling hydrogels for pharmaceutical and biomedical applications. *Int J Pharm*. 2008; 355:1–18. [PubMed: 18343058]
196. Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *J Clin Endocrinol Metab*. 1987; 64:51–58. [PubMed: 3782436]
197. Paavola A, Tarkkila P, Xu M, Wahlstrom T, Yliruusi J, Rosenberg P. Controlled release gel of ibuprofen and lidocaine in epidural use--analgesia and systemic absorption in pigs. *Pharm Res*. 1998; 15:482–487. [PubMed: 9563081]
198. Paavola A, Yliruusi J, Kajimoto Y, Kalso E, Wahlstrom T, Rosenberg P. Controlled release of lidocaine from injectable gels and efficacy in rat sciatic nerve block. *Pharm Res*. 1995; 12:1997–2002. [PubMed: 8786979]
199. Desai SD, Blanchard J. In vitro evaluation of pluronic F127-based controlled-release ocular delivery systems for pilocarpine. *J Pharm Sci*. 1998; 87:226–230. [PubMed: 9519158]
200. Johnston TP, Palmer WK. Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. *Biochem Pharmacol*. 1993; 46:1037–1042. [PubMed: 8216346]
201. Jeong B, Bae YH, Kim SW. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. *Journal of Controlled Release*. 2000; 63:155–163. [PubMed: 10640589]
202. Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, Leroux JC, Atkinson BL, Binette F, Selmani A. Novel injectable neutral solutions of chitosan form biodegradable gels in situ. *Biomaterials*. 2000; 21:2155–2161. [PubMed: 10985488]
203. Johnston TP, Punjabi MA, Froelich CJ. Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice. *Pharm Res*. 1992; 9:425–434. [PubMed: 1614979]
204. Jeong B, Bae YH, Kim SW. Thermoreversible gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions. *Macromolecules*. 1999; 32:7064–7069.
205. Choi SW, Choi SY, Jeong B, Kim SW, Lee DS. Thermoreversible gelation of poly(ethylene oxide) biodegradable polyester block copolymers. II. *J Polym Sci Pol Chem*. 1999; 37:2207–2218.
206. Packhaeuser CB, Schnieders J, Oster CG, Kissel T. In situ forming parenteral drug delivery systems: an overview. *European Journal of Pharmaceutics and Biopharmaceutics*. 2004; 58:445–455. [PubMed: 15296966]
207. Byun EB, Sung NY, Kim JH, Choi JI, Matsui T, Byun MW, Lee JW. Enhancement of anti-tumor activity of gamma-irradiated silk fibroin via immunomodulatory effects. *Chem Biol Interact*. 2010; 186:90–95. [PubMed: 20338156]
208. Kojthung A, Meesilpa P, Sudatis B, Treeratanapiboon L, Udomsangpetch R, Oonkhanond B. Effects of gamma radiation on biodegradation of Bombyx mori silk fibroin. *Int Biodeter Biodegr*. 2008; 62:487–490.

209. Ishida K, Takeshita H, Kamiishi Y, Yoshii F, Kume T. Radiation degradation of silk. JAERI-Conf, 2001-005. 2001:130–138.
210. Mandal BB, Kundu SC. A novel method for dissolution and stabilization of non-mulberry silk gland protein fibroin using anionic surfactant sodium dodecyl sulfate. Biotechnol Bioeng. 2008; 99:1482–1489. [PubMed: 17969177]
211. Lawrence BD, Omenetto F, Chui K, Kaplan DL. Processing methods to control silk fibroin film biomaterial features. J Mater Sci. 2008; 43:6967–6985.
212. Kundu J, Poole-Warren LA, Martens P, Kundu SC. Silk fibroin/poly(vinyl alcohol) photocrosslinked hydrogels for delivery of macromolecular drugs. Acta Biomater. 2012

Figure 1. Silk Fibroin constructs for delivery of skeletal therapeutics

Cartoon describing the sequential process of growth factor deposition on SF spun mats. A: A first blend containing IGF-I/TGF-β3 was spun (A; green). B: The target was rotated and another SF solution containing BMP-2 (2nd blend) was spun. Disks (circles in B; ~ 2cm diameter) were punched from regions I (containing the 1st blend), III (containing the 2nd blend) and the intermediate zone II containing all three growth factors. Please note, that blends containing other growth factors were spun and tested as well. Human mesenchymal stem cells (hMSC) were seeded on these disks and cultured for weeks using a single medium supporting both, chondrogenic and osteogenic differentiation. C; D: Glucose consumption of hMSC over time on differentially loaded disks. E; F: calcium (μg) and GAG (μg) deposition per disc after 3 weeks of culture. The growth factors blended into the SF scaffold are detailed on the abscissa. Bars indicate statistical differences ($p < 0.05$) compared to scaffolds

loaded with (1) TGF- β 3, (2) BMP-2/ TGF- β 3, (3) BMP-2, (4) control (no GF), (5) TGF- β 3/ IGF-I and (6) BMP-2/TGF- β 3/IGF-I (modified from [129]).

Table 1

Overview of parenteral, injectable drug delivery systems*

Injectable drug delivery system	Physical principle of in situ solidification	Advantages	Disadvantages	Literature
Thermoplastic pastes	Injection of a heated liquid – transformed to a solid upon cooling to body temperature	<ul style="list-style-type: none"> • Excellent injectability 	<ul style="list-style-type: none"> • Burst release • Drug activity • Drug stability • Scar formation • Necrosis • Alcohols as initiators • Low drug load 	[151-156]
In situ cross linked systems	Thermal (T) Photocrosslinked (P) Ion mediated (I)	<ul style="list-style-type: none"> • T: Obsolete • P: Rapid • Low burst release 	<ul style="list-style-type: none"> • T: Incompatible due to high heat • P: Inelasticity (brittleness) of implant • P: Less suited for molecular weight drugs • I: Burst release • I: Slow degradation 	T: [185-187] P: [188-193] I: [194, 195]
In situ polymer precipitation	Injection of water insoluble polymers – precipitation when in contact with body fluids	<ul style="list-style-type: none"> • FDA approval • Up to 6 months (or even 12 months) release • Low manufacturing costs 	<ul style="list-style-type: none"> • Burst release • Organic solvents can cause tissue reaction • Volume restriction for application sites • Viscosity hampers administration ease • γ-irradiation required for sterilization 	[163-167, 196]
Thermally induced gels	Temperature dependent sol – gel transition	<ul style="list-style-type: none"> • No organic solvents • 4-8 week sustained release possible 	<ul style="list-style-type: none"> • Lack of toxicity data • Phase separation • Low drug load 	[168-171, 197-205]

Injectable drug delivery system	Physical principle of in situ solidification	Advantages	Disadvantages	Literature
Gels from hybrid-polymers	Coiled protein assembly (in response to pH or temperature changes)	<ul style="list-style-type: none"> No organic solvents No heat 	<ul style="list-style-type: none"> Lack of toxicity data Possible immune-reaction Burst Release 	[172, 173]
SF based injectable systems **	Conformational change into β -sheet	<ul style="list-style-type: none"> Aqueous based system Sterilization (filtration or heat) Potentially low COGS Protection of therapeutic protein stability (likely requiring no further excipients) High drug load and release profiles for weeks (for hydrophobic and protein therapeutics as well as weak bases) 	<ul style="list-style-type: none"> Unknown toxicity profile of subcutaneous SF Many unknowns due to the novelty of the approach **. 	[71, 72, 182, 184]

* modified from [150, 206],

** Table 2 for further details

Table 2

Open challenges for the development of SF injectable systems. A ‘requirement’ represents a development need for an injectable system before it can be used in clinical trials; a ‘potential hurdle’ is an anticipated challenge to overcome the respective requirement. Citations are provided where guiding information is available.

	Requirement	Potential hurdle
Presentation image of injectable system	<ol style="list-style-type: none"> 1 Liquid form may be stable for up to two years at room temperature if properly formulated and stored (development required – long term stability data unpublished) 2 Solid form may be feasible through freeze drying, a standard process for SF 	<ol style="list-style-type: none"> 1 Physical tampering of the liquid image may result in quality issues with storage time (continuously increasing β-sheet content) 2 Reconstitution of freeze dried SF in aqueous solution may be challenging. This is typically done in organic solvents (e.g. hexafluoroisopropanol – HFIP) today which is not biocompatible [6].
Sterilization	<ol style="list-style-type: none"> 1 Liquid: Excellent resistance to heat with at least autoclaving being an amenable process (blending with protein therapeutics after SF sterilization under aseptic conditions) [51] 2 Solid: Resistance to / toxicity as a result of γ- radiation need to be explored before final sterilization of a dried SF / protein drug blend can be used [207] 	<ol style="list-style-type: none"> 1 Liquid: none 2 Solid: uncontrollable chain break during radiation process [208, 209]
Processing	Stability at room temperature [210].	Change in β -sheet content / physical properties during pumping and mechanical stress [211]
Protein therapeutic release profile	<p>Sustained release profile demonstrated with potential to extend to several months [212]</p> <p>Excellent loading possibilities [183]</p>	<p>Change of release properties with storage impacting shelf life</p> <p>Unknown bioavailability (strong binding of protein therapeutics to SF after implantation potentially resulting in incomplete release – indirect evidence from where SF [43]films were loaded with Lysozyme)</p>
Toxicity	Good biocompatibility for implanted SF scaffolds and in vitro [15, 68, 70]	Unknown safety profile for subcutaneous / i.m. administration for injectable systems may lead to local irritations although evidence points to a benign profile [68]