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Fibers with nanoscale diameters supply a benefit because of the high surface area for biomaterial scaffolds. Electrospun silk fibroin-based fibers prepared from aqueous silkworm silk solutions. The ability of electrospun silk matrices to support BMSC attachment, growth and spreading, combined with a biocompatibility and biodegradable properties of the silk protein matrix, result shown suitable biomaterial matrices as scaffolds for tissue engineering [3]. Scaffold configuration, composition, and resulting properties which it is effects into tissue development. The influence of silk fibroin concentration processing method and three-dimensional scaffold structure on bone tissue formation by osteogenic differentiation of human adipose tissue derived stem cells (hASC). It is resulted in a very similar to bone tissue that was formed in all silk fibroin scaffold groups. [4]A silk-fiber matrix studied as a suitable material for tissue engineering anterior cruciate ligaments (ACL). The matrix designed to match the complex and demanding mechanical requirements of a native human ACL, involving adequate fatigue performance. The results support the conclusion that properly prepared silkworm fiber matrices, aside from giving unique benefits in terms of biocompatibility and slow degradability as well as mechanical properties , can provide suitable biomaterial matrices for the support of adult stem cell differentiation toward ligament lineages. These results point toward this matrix as a new option for ACL repair to overcome current limitations with synthetic and other degradable materials. [5] Silk fibroin (SF) and elastin (EL) scaffolds were produced for the first time for the treatment of burn wounds. The self-assembly properties of SF, together with the excellent chemical and mechanical stability and biocompatibility, were combined with elastin protein to produce scaffolds with the ability to mimic the extracellular matrix (ECM). Porous scaffolds were obtained by lyophilization and were further crosslinked with genipin (GE). All results indicated that the composition of the scaffolds had a significant effect on their physical properties, and that can easily be tuned to obtain scaffolds suitable for biological applications. Wound healing was assessed through the use of human full-thickness skin equivalents (EpidermFT. The cytocompatibility demonstrated with human skin fibroblasts together with the healing improvement make these SF/EL scaffolds suitable for wound dressing applications. [6]This study describes the developmental physicochemical properties of silk fibroin scaffolds derived from 26 high-concentration aqueous silk fibroin solutions. The silk fibroin scaffolds were prepared by leaching and freeze- 28 drying methodologies. The results indicated that the antiparallel b-pleated sheet (silk-II) conformation 29 was present in the silk fibroin scaffolds. All the scaffolds possessed a macro/microporous structure. Based on these results, the scaffolds developed in this study are proposed to be suitable 39 for use in meniscus and cartilage tissue-engineered scaffolding. [7] Bone morphogenetic protein (BMP)-2 has a very important role in bone regeneration and formation. So, the ability to immobilize this molecule in certain matrices in bone tissue engineering. Using carbodimide chemistry, BMP-2 was immobilized on silk fibroin films. Whereas human bone marrow stromal cells cultured on unmodified silk fibroin films in the presence of osteogenic stimulants exhibited little if any osteogenesis, the same cells cultured on BMP-2 decorated films in the presence of osteogenic stimulants differentiated into an osteoblastic lineage as assessed by their significantly elevated alkaline phosphatase activity, calcium deposition, and higher transcript levels of collagen type I, bone sialoprotein, osteopontin, osteocalcin, BMP-2, and cbfa1. The results explain that BMP-2 covalently coupled on silk biomaterial matrices retains biological function in vitro based on the induction of osteogenic markers in seeded bone marrow stromal cells.[8] Silks are reassessed as biomaterial scaffolds. We report on the covalent decoration of silk films with integrin recognition sequences (RGD) as well as parathyroid hormone (PTH, 1–34 amino acids) and a modified PTH 1–34 (mPTH) involved in the induction of bone formation. Calcification was also significantly elevated on RGD compared to the other substrates with an increase in number and size of the mineralized nodules in culture. Thus, RGD covalently decorated silk appears to stimulate osteoblast-based mineralization in vitro. [9] This study was to check biocompatibility effect on bone regeneration, and to reform the biocompatibility of the SF Nano fiber membrane. The SF Nano fiber membrane was shown to have a suitable biocompatibility with enhanced bone regeneration and no evidence of any inflammatory reaction. The results shown that the SF membrane very important for bone regeneration and should be useful like guider for bone regeneration.[10] the influence of silk fibroin concentration has an adjusted (6 or 17%) and three-dimensional scaffold structure (lamellar or porous, with distinct pore size) on bone tissue formation by osteogenic differentiation of human adipose tissue derived stem cells (hASC) and correspondent processing method (aqueous or HFIP-derived). The result was shown that very similar bone tissue was formed in all silk fibroin scaffold groups, evaluated by alkaline phosphatase activity, calcium production, collagen type I deposition and scaffold bone volume fraction.[11]A novel biomimetic design of the SF-based nerve graft (SF graft) was developed which was composed of a SF-nerve guidance conduit (NGC) inserted with oriented SF filaments. The examined functional and morphological parameters show that SF grafts could promote peripheral nerve regeneration with effects approaching those produced by nerve auto grafts which are generally considered as the gold standard for treating large peripheral nerve defects, thus raising a potential possibility of using these newly developed nerve grafts as a promising alternative to nerve auto grafts.[12] Rat dorsal root ganglia (DRG) was cultured on the substrate made up of silk fibroin fibers and observed the cell outgrowth from DRG during culture by using light and electron microscopy coupled with immunocytochemistry. On the other hand, we cultured Schwann cells from rat sciatic nerves in the silk fibroin extract fluid and examined the changes of Schwann cells after different times of culture these data indicate that silk fibroin has good biocompatibility with DRG and is also beneficial to the survival of Schwann cells without exerting any significant cytotoxic effects on their phenotype or functions, thus providing an experimental foundation for the development of silk fibroin as a candidate material for nerve tissue engineering applications.[13] Nerve conduits (NC) for peripheral nerve repair should guide the sprouting axons and physically protect the axonal cone from any damage. The NC should also degrade after completion of its function to obviate the need of subsequent explanation and should optionally be suitable for controlled drug release of embedded growth factors to enhance nerve regeneration. Silk fibroin (SF) is a biocompatible and slowly biodegradable biomaterial with excellent mechanical properties that could meet the above stated requirements. SF material (films) supported the adherence and metabolic activity of PC12 cells and in combination with nerve growth factor (NGF), supported neurite outgrowth during PC12 cell differentiation. This study encourages the further exploitation of SF-NC for growth factor delivery and evaluation in peripheral nerve repair. [14]The potential of silk fibroin and chitosan blend (SFCS) biological scaffolds was investigated for the purpose of applications in tracheal tissue reconstruction with cartilage tissue engineering. cartilage generation on engineered chondrocyte–scaffold constructs with and without a perichondrium wrapping was tested and The capability of these scaffolds as cell carrier systems for chondrocytes was determined . Result shown in a tracheal transplant with properties which it is similar to those of the fully functional native trachea. [15]Biodegradable polymer, poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx), used to fabricate the tissue engineering as cardiovascular scaffolds because of its suitable mechanical properties and also controllable. Silk fibroin (SF) with no blood clotting, low inflammation and good cell and tissue compatibility in vitro and vivo is adopted as a surface modificator to improve the biocompatibility of PHBHHx. The adhesion of SF on PHBHHx surface was investigated. Silk fibrion modified PHBHHx scaffolds have very good biocompatibility with cardiovascular related cells, that is mean its potential help for the extensive applications of PHBHHx in the cardiovascular regeneration.[16] Cell affinity is one of the important issues required for developing tissue engineering materials. Although the poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) has been attractive for its controllable mechanical properties recent years, its cell affinity is still necessary to be improved for the requirements. For this purpose, the regenerated silk fibroin (SF) was coated on the PHBHHx films and its porous scaffolds the SF modified PHBHHx material is maybe a potential material applicable in the cardiovascular tissue engineering.[17] Currently synthetic grafts demonstrate moderate success at the macro vascular level, but fail at the micro vascular scale (<6 mm inner diameter). We report on the development of silk fibroin microtubes for blood vessel repair with several advantages over existing scaffold materials designs. These microtubes were prepared by dipping straight lengths of stainless steel wire into aqueous silk fibroin, where the addition of poly(ethylene oxide) (PEO) enabled control of microtube porosity. These results suggest that silk microtubes, either implanted directly or preseeded with cells, are an attractive biomaterial for micro vascular grafts.[18 ] the core silk fibroin fibers have a high biocompatibility in vitro and in vivo by comparable with other most commonly used biomaterials such as collagen and polylactic acid . as well as , the unique mechanical properties of the silk fibers, the variety of side chain chemistries for ‘ decoration’ with adhesion factors and growth, and the additional rationale supported by genetically tailor and the protein provide for the exploration of this family of fibrous proteins for biomaterial applications. Studies about silks to address biomaterial and matrix scaffold were focused on silkworm silk. With the variety of silk-like fibrous proteins from spiders and insects, a range of native or bioengineered variants can be expected for application into different junctions of clinical needs. [19]Color dye-doped silk fibroin nanoparticles fabricated using a micro emulsion method. Yielding color dye-doped silk fibroin nanoparticles, 167 nm in diameter. The secondary structure of nanoparticles showed a β-sheet conformation. the size and size distribution were measured, The morphology of nanoparticles was determined. The observed stability of the loaded fluorescent molecules in the silk fibroin nanoparticles showed that it can be used as new and important devices for molecular imaging and bioassays. The slow degradation of silk combined with its biocompatibility, and Because of the Nano-scale size, their capacity to encapsulate fluorescent dye, they may a have a great impact and more use in various biological applications. [20] Obtaining microspheres and/or submicronic particles by spray dryer method was developed or completed with transition from the random coil to the b-sheet structure during spray dryer treatment. It defined by The various pH range of SFMP\_s swelling ratio is dependent on the pH of the solution, not on the occurred gelation. Morphologically, SFMP was spherical in shape, and particles, average 2 \_ 10 lm in size. The characteristic structure might be applied to immobilization of drugs. SFMP would be used for the biomaterials with skin affinity, is superior to other matrix materials [21] Cross-linked and non-cross-linked silk fibroin (SF) microspheres using the simple water-in-oil emulsion solvent diffusion method was studied. SF microparticles were spherical in shape and smooth in surface. SF microsphere sizes were found to depend upon various process parameters. Both non-cross-linked and genipin-cross-linked SF microspheres contained porous structures.. The genipin cross-linking induced SF conformational transition from random coil to β-sheet form but the shape and size of the SF microparticles not change. These SF microspheres might be suitable microcarriers for hydrophilic drug delivery.[22]A novel solution-enhanced dispersion by supercritical CO2 (SEDS) was employed to prepare silk fibroin (SF) nanoparticles. The resulting SF nanoparticles exhibited a smooth surface, a spherical shape and a narrow particle size distribution with a mean particle diameter approximately 50 nm nanoparticles before and after ethanol treatment indicated conformation transition of SF nanoparticles from random coil to b-sheet form and thus water insolubility SF nanoparticles after ethanol treatment imposed no toxicity. The (indomethacin) IDMC–SF nanoparticles after ethanol treatment showed a significantly sustained release over 2 days. These studies of SF nanoparticles shown the suitability of the SF nanoparticles prepared by the SEDS process as a biocompatible carrier to deliver drugs and also the feasibility of using the SEDS process to arrive the goal of co-precipitation of drug and SF as composite nanoparticles for controlled drug delivery. [23]Biologically derived nanoparticles (100 nm) were fabricated for local and sustained Therapeutic curcumin delivery to cancer cells. SF-derived curcumin nanoparticles show higher efficacy against breast cancer cells and have the potential to treat in vivo breast tumors by local, sustained, and long-term therapeutic delivery as a biodegradable system. [24] Biologically derived delivery systems offer promise in this regard owing to minimization of adverse effects while increasing the efficacy of the entrapped therapeutic. Silk fibroin nanoparticles overcome barriers set by synthetic non degradable nanoparticles made of silicone, polyethylene glycol and degradable polylactic acid–polyglycolic acid polymers. Silk fibroin-mediated delivery has demonstrated high efficacy in breast cancer cells. While the targeting is associated with the specificity of entrapped therapeutic for the diseased cells, silk fibroin-derived particles enhance intracellular uptake and retention resulting in down modulation of more than one pathway due to longer availability of the therapeutic. The mechanism of targeting for the nanoparticle is based on the silk fibroin composition, b-sheet structure and self-assembly into b-barrels. [25]Drug-loaded SF spheres with and without polyethylene glycol diglycidyl ether (PEGDE) crosslinking were prepared by a water-in-oil emulsion solvent diffusion method. Effects of homogenizing speed and PEGDE ratio on characteristics and drug release behaviors of the SF spheres were determined. The results suggested that the desired drug release profiles of SF spheres can be design by adjusting the particle size and PEGDE ratio. [26] Silk fibroin was conjugated with methoxypoly(ethylene glycol) derivatives to prepare silk nanoparicles. Conjugation of SF with PEG was examined with various instrumental analyses. Nuclear magnetic resonance spectrometry and amino acid analysis showed that serine and tyrosine residues in SF were reacted with PEG and resulted in increasing molecular weight. The sizes and shapes of SF nanoparticles observed by transmission electronmicroscope were ranged about 150-400 nm in diameter and spherical morphology. UV/VIS spectrometry showed SF nanoparticles might be outer PEG and inner SF structure. [27] The goal of this proof-of-concept study was the fabrication of drug-loaded silk fibroin (SF) spheres under very mild processing conditions. The spheres were fabricated using the laminar jet break-up of an aqueous SF solution, which was induced by a nozzle vibrating at controlled frequency and amplitude. SF particles were spherical in shape as determined by SEM with diameters in the range of 101 µm to 440 µm, depending on the diameter of the nozzle and the treatment to induce water insolubility of SF. These results favor further investigation of SF spheres as a platform for the controlled release of sensitive biologicals. [28]The objective of the present study is to investigate the possibility of preparing pure protein microspheres from regenerated silk fibroin (RSF). It is found that RSF microspheres, with predictable and controllable sizes ranging from 0. 2 to 1. 5 µm, can be prepared via mild self-assembling of silk fibroin molecular chains. The results show that the particle size and size distribution of RSF microspheres are greatly affected by the amount of ethanol additive, the freezing temperature and the concentration of silk fibroin. Finally, the mechanism of RSF microspheres formation is also discussed based on our experimental results.[29]We investigated the biomaterial and pharmaceutical utility of pure silk fibroin (SF) protein as a possible for separation, using Sephadex G-25 gel filtration chromatography and simply preparing SF microsphere particles (SFMP) by spray dryer. Also, some of its physicochemical properties and morphology were investigated. Obtaining microspheres and/or submicronic particles by spray dryer method was accelerated or completed with the transition from the random coil to the b-sheet structure during spray dryer treatment. It was identified by the basic Fourier transform infrared spectroscopy of SFMP. The various pH range of SFMPs swelling ratio is dependent on the pH of the solution, not on the occurred gelation.. The average molecular weight (MW) of pure SF protein dissolved in calcium chloride is about 61, 500 g/mol as measured by gel permeation chromatography.[30] The objective of this work was to prepare SF spheres for drug delivery application. Drug-loaded SF spheres with and without polyethylene glycol diglycidyl ether (PEGDE) crosslinking were prepared by a water-in-oil emulsion solvent diffusion method. The results suggested that the desired drug release profiles of SF spheres can be tailored by adjusting the particle size and PEGDE ratio.[31] The pharmaceutical utility of silk fibroin (SF) materials for drug delivery was investigated. SF films were prepared from aqueous solutions of the fibroin protein polymer and crystallinity was induced and controlled by methanol treatment. Dextrans of different molecular weights, as well as proteins, were physically entrapped into the drug delivery device during processing into films. Drug release kinetics were evaluated as a function of dextran molecular weight, and film crystallinity. In conclusion, SF is an interesting polymer for drug delivery of polysaccharides and bioactive proteins due to the controllable level of crystallinity and the ability to process the biomaterial in biocompatible fashion under ambient conditions to avoid damage to labile compounds to be delivered. [32] The controlled release of fluorescein-iso-thio-cyanate (FITC)-labeled dextrans from methanol-treated and untreated silk fibroin films was modeled to characterize the release kinetics and mechanisms. A linear regression was fit to the relationship between molecular weight and the percent of entrapped FITC-dextran particles. Using these defined linear relationships, we present an updated version of the diffusion model for simulating release of FITC-dextran of varied molecular weights from methanol-treated and untreated silk films. [33]

## Films

Robust ultrathin multilayer films of silk fibroin were fabricated by spin coating and spin-assisted layer-by-layer assembly and their mechanical properties were studied both in tensile and compression modes. The superior toughness is many times higher than that usually observed for usual polymer composites. These exceptional properties are caused by the highly crystalline b-sheets, serving as reinforcing Fillers and physical crosslinks, a process that is well known for bulk silk materials but it is shown here to occur in ultrathin Films as well, even with their limited dimensions. However, the confined state within films thinner than the lengths of the extended domains causes a significantly reduced elasticity which should be considered in the design of nanosized films from silk Materials. Such regenerated silk fibroin films with exceptional mechanical strength have potential applications in microscale Biodevices, biocompatible implants, and synthetic coatings for artificial skin. [34]