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Silk Biotechnology

A New Spin

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Silk-Based Biomaterials

Fibre of Fitness

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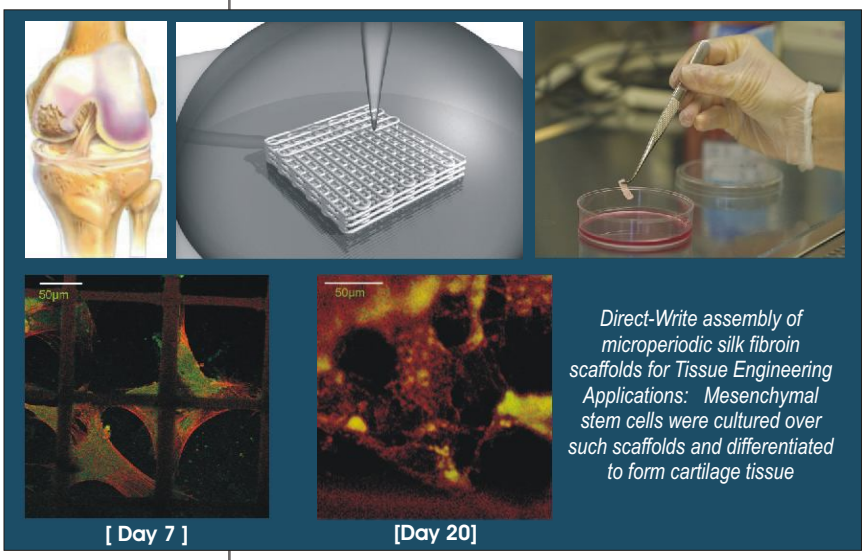
Current generation therapeutic modalities are gradually moving towards personalized and rationally targeted strategies. Urged by this need for developing engineered tissue micro-environment for individual patients, Tissue engineering (TE) based biotechnologies have emerged as an approach with the potential to revolutionize the traditional concept of healthcare systems around the world. TE envisages building up patient-specific human tissues in laboratory, and then transplanting them into patients suffering from tissue damage due to disease, accident or congenital abnormalities. In short, a few cells can be collected from a patient, characterized and cultured over a strategically designed polymeric architecture ("scaffold") within a bioreactor and can be implanted back in the area of defect, once the engineered tissue has matured.



Direct-Write assembly of microperiodic silk fibroin scaffolds for Tissue Engineering Applications: Mesenchymal stem cells were cultured over such scaffolds and differentiated to form cartilage tissue

Many biomaterials have already been explored as polymeric scaffolds for TE applications. These include alginate, collagen, chitosan, polyglycolic or polylactic acids. However, none of these satisfy all the criteria of a functional scaffold system. In this context, silk is an attractive biomaterial as it can address these needs due to its following properties:

a) Biocompatibility: Silks have been used as surgical sutures for decades, are biocompatible and are also less immunogenic and inflammatory than collagens or polyesters such as polyglycolic or polylactic acids.

b) Stability and Mechanical Properties: Silks exhibit remarkable strength, toughness and compressive modulus which exceeds that of other commonly used degradable polymeric biomaterials. Moreover, due to their excellent thermal stability, silks can be autoclaved without loss of mechanical integrity. Unlike many other polymeric biomaterials, silks achieve these outcomes without any chemical or photo-initiated cross-linking. They are stabilized by beta-sheet secondary structures which are physical crosslinks formed via hydrogen bonding and hydrophobic interactions via inter and intra chain interactions.

c) Purity: Unlike bovine-derived collagens, silks have no known bioburdens and with long-term experience no evidence for this type of contamination has been reported.

d) Modifiable: Silk fibroin can be easily modified with selective cell binding or activation features. The use of unmodified silk fibroin, which does not contain cell-binding domains and is predominately hydrophobic, suffices for culturing many cell types. However, chemical decoration with cell binding peptides and other cell modulating factors has been reported using facile carbodiimide coupling, providing options to functionally modify the protein for selective tissue-specific needs.

e) Controlled Degradability: Silks can be designed to degrade relatively fast (weeks) to very slow (years) depending on the mode of processing, beta-sheet content and material format.

These attributes are mostly responsible for the remarkable functional performance of silk scaffolds for TE interventions.

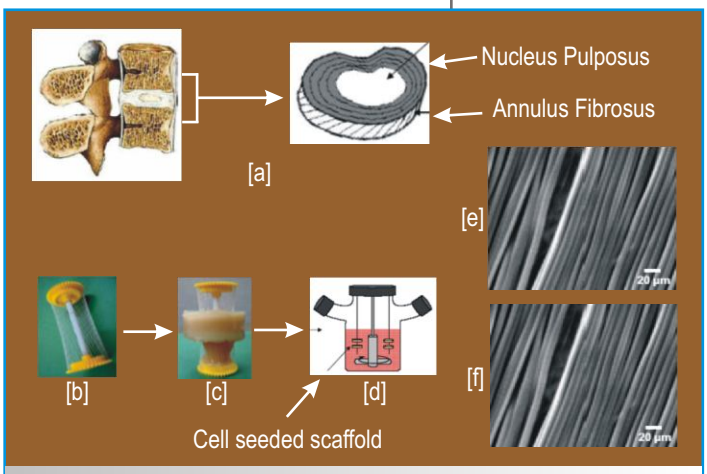
Another important attribute of silk is that it offers unique mechanical properties in different material formats. Along with properties attainable with silk protein polymers as mentioned above, improved insights into fundamental structure-function relationships of such architectures can play a crucial role in tissue engineering. Prof David Kaplan's group at the Tufts University, Boston (USA) has fabricated silk scaffolds in a wide range of shapes and architecture, mostly by utilizing textile technologies. A wire rope design was developed using textile engineering techniques to generate biomaterial replacements for ligaments having mechanical strength equivalent to the human Anterior Cruciate Ligament. Human bone marrow-derived stem cells and fibroblasts from ligament seeded on silk fibroin wire ropes could not only be attached and proliferated, but when cells were exposed to mechanical stimulation in novel bioreactor, expressed collagen types I, III and tenascin C as typical characteristics of human ligaments. Based on these studies SeriACL™ Graft, developed by Serica Technologies, Inc. (USA) has demonstrated a definitive evidence of ligament regeneration in small and large scale animal clinical trials. This ▶▶

can lead to a dramatic improvement in ligament surgical outcomes as well as a significant decrease in healthcare costs.

Non-woven mats are of great interest as scaffolds due to their high *surface area* to *volume* ratio and rougher topography *(*needed for cell attachment*)*. Silk fibroin has been used to generate non-woven silk matrix from regenerated native silk fibers (macro-diameter fiber) as well as by electrospinning (nano-fibers). Non-woven silk fibroin mats can be prepared by partial dissolution of native silk fibers, followed by processing using needle-punching or hydro-entanglement techniques. When such mats were implanted subcutaneously or ectopically in animal models, they demonstrated good biocompatibility. Histology, mRNA transcript levels, and immuno-histochemistry studies have suggested that the silk fibroin non-woven mat could guide formation of various targeted tissue architectures, if provided the correct impetus. Silk fibroin polyethylene oxide blends have been electrospun into nano-diameter fibers for delivering cell morphogens, like bone morphogenetic protein-2 which induces osteogenesis from mesenchymal stem cells. Nanoparticles of hydroxyapatite were added with, or without BMP-2 and the mats were seeded with human mesenchymal stem cells and grown under osteogenic conditions. The silk fibroin mats supported the differentiation of stem cells to osteoblast-like cells, and the mats formed with hydroxyapaptite and BMP-2 showed the highest level of calcium deposition and upregulation of osteogenic markers. Electrospun mats with hydroxyapatite also induced significant osteogenesis and the upregulation of BMP-2 transcript, comparatively in lower extent. Silk fibroin non-woven mats electrospun from a 98% silk-formic acid solution were implanted in calvarial defects of rabbits for bone regeneration and resulted in complete healing with new bone at 12 weeks.

Regenerated silk fibroin solutions, both aqueous and organic solvent-based, have been used to prepare porous sponges scaffolds. Sponges have been formed using porogens (e.g., sodium chloride or sugar granules), gas foaming and lyophilization. A gradient of pore sizes can be generated by stacking porogens of different sizes before pouring into a mould. Sponges with varying porosity can be controlled by stacking variations of salt / HFIP silk solutions. Aqueous silk fibroin sponges demonstrated improved cell attachment than organic solvent-based porous sponges, probably due to rougher surfaces. Aqueous silk sponges with high porosity and mechanical strength similar to bone can be obtained. Stiffness, compressive strength and modulus can be elevated with an increase in percent silk fibroin solution utilized in the process. Enzymatic degradation of aqueous-based sponges was more rapid than solvent-based sponges. Aqueous silk fibroin sponges seeded with chondrocytes showed faster proliferation and produced higher content of glycosaminoglycan compared with collagen sponges, demonstrating feasibility of cartilage tissue engineering. Porous silk fibroin scaffold sponges seeded with rabbit chondrocytes and cultured in chondrogenic media yielded a frictional coefficient similar to that of native cartilage after 28 days of culture.

Prof. C. James Kirkpatrick's group from Mainz (Germany) cultured endothelial cells (both primary and transformed) on silk fibroin mats. Cell attachment and proliferation greatly improved with a coating of collagen type-I or fibronectin, due to presence of RGD sequences for cell binding. Endothelial cells cultured for only a week formed microvessel like structures on non-woven mats. Prof. David Kaplan's group developed small diameter vascular grafts from both, cylindrical shaped silk fibroin hydrogel as well as from electrospun tubular nanofibrous scaffolds. Mechanical parameters of such tubes demonstrated burst strength sufficient to withstand arterial pressures and tensile properties comparable to native blood vessels of similar diameter range.



Rapid prototyped Silk scaffolds for Tissue engineering of Intervertebral disc (a) Physiology of Intervertebral disc, (b) Degummed Silk Fibrous aligned scaffold, (c) Silk fibre-hydrogel composite construct, (d) Culture in spinner flask bioreactor, (e, f) Cells following fibre orientation & alignment

Recently, at IIT- Delhi, we have reported microperiodic silk "direct write" scaffolds for fabricating patient-specific complex tissue construct (*see picture on page 10*). As soon the patient arrives at the hospital with any injury or disease, the site of injury can be scanned, and custom-made "direct write" scaffolds can be fabricated with precise control to have a perfect fit to the specific defect site. The development of controlled fibrous architectures has the potential to help in generating a more fundamental understanding of the role of architectural complexity on tissue development and remodeling, as well as the formation of multicellular aggregates for the complex micro-environments of targeted tissue.

During the last decade, TE research has made significant progress towards the design of tissue constructs to repair or replace lost morphology and functions in diseased or damaged organs. The next paradigm shift in this context would

(*Contd. on page 30*)

Fibre of Fitness...(contd. from page 11)

be to establish simple in-vitro disease model systems, which mimic their in-vivo counterparts, to gain insights into mechanisms of disease origins, pathological conditions and to screen treatment options, by combining tissue engineering principles and knowledge from engineering. Using state-of-the-art textile engineering facilities established at the Indian Institute of Technology, New Delhi, our group is currently focusing on development of such "in vitro disease model systems". These offer the potential to advance our understanding of human disease pathology related processes, including providing enhanced understanding of intra- and inter-cellular signaling pathways in order to develop novel treatment modalities.

Due to an aging population across the world, millions of elderly people worldwide are suffering from severe lower back pain, due to degenerative intervertebral disc related problems. Intervertebral disc has a complex architecture, consists of three tissue components: Annulus Fibrosus (AF), Nucleus Pulposus (NP) and cartilage end-plates. The AF has a series of loosely connected concentric layered and laminated structure and each layer is reinforced by a highly oriented pattern of mainly collagen (type-I and II) fibers. In these lamellae, the collagen fibers lie parallel to each other at an angle of approximately 60° to the spine axis and are oriented in opposite directions in successive layers *(See graphic on page11)*. With increasing age, AF herniates through the gradually dehydrating NP, causing intense pressure and pain. Most commonly used treatment modality is only symptomatic pain relief by drugs, instead of identifying underlying biological mechanisms. Alternatively surgical correction of intervertebral disc involves spine fusion, removal of defective disc tissue and total disc replacement with prosthetic implant can be done; all of which are associated with some limitations.

One major focus of our group is to develop bioengineered intervertebral disc using a fibre-hydrogel composite construct. The construct is fabricated by combining (a) rapid prototyped aligned silk fibroin scaffold where Silk fibres are aligned at 45-60 degree angle, and the next layers are laid in alternate direction keeping same angle, to simulate the gross morphology of AF lamellar architecture, and (b) a novel hydrogel material having covalently crosslinked silk fibroin and other chondrogenic biopolymers, which stabilizes the whole fibrous structure. The implementation of this study might set the stage for fabricating the novel fibre-hydrogel composite scaffold which may have targeted and localized expression of fibro-cartilage or hyaline cartilage phenotype in the same construct. This engineered tissue construct can be implanted in the area of defect in patients spinal disc, who are suffering from degeneration of Intervertebral disc. This in vitro model can also be used to establish a 'disease model system' to get valuable insights into genetic modulations responsible for development of degenerative intervertebral disc.

In a nutshell, silk can help to meet the growing need for highly specialized biomaterials in Tissue Engineering & Regenerative Medicine. It is tempting to speculate that in near future these engineered human tissue models may provide a new way to study disease in- vitro, and help in designing patient-specific treatments under precisely controlled conditions. This outcome would also fill a critical gap that presently exists between cell-based assays and human clinical trials.■

Options for Improvement ...(contd. from page 19)

Mulberry is a high water demanding crop, requiring as high as 500-700 liters of water to produce 1 kg of fresh leaf. As a result, leaf quality and yield are extremely low in predominantly rainfed areas. The major research agenda thus has been to improve quality yield per unit of water input. In addition, foliage productivity and quality are also affected by specific diseases and nutrient deficiencies.

Molecular Breeding-An Appropriate Option

Most of the traits that improve biotic and abiotic stress tolerance are multigene controlled and are quantitatively inherited. Therefore, yield improvement is best achieved through reshuffling favorable alleles to bring together several traits of agronomic relevance into a single genetic background. Though high throughput and accurate phenotyping strategies have been developed and standardized, introgressing these traits through conventional approaches is difficult. Intervention of DNA marker based technology in identifying trait introgressed lines is expected to significantly augment breeding efforts in introgressing complex physiological traits.

Development of molecular breeding strategies requires species-specific genomic resources, construction of genetic linkage maps and finally the QTL maps. Accomplishments in this direction are not yet impressive. However, pioneering work has been initiated by Center for Cellular and Molecular Biology (CCMB) Hyderbad in▶▶