BIOPRINTED SILK FOR HYPERTROPHY INHIBITION AND CARTILAGE REGENERATION

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by

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Submitted

In fulfilment of the requirements of the degree of Doctor of Philosophy

to

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Dedicated to my parents...

Mr. Chandidas Majumder and

Mrs. Mithu Majumder

for their infinite love, support, and encouragement

"It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change."

- Charles Darwin

CERTIFICATE

This is to certify that the thesis entitled ***"Bioprinted Silk for Hypertrophy Inhibition and Cartilage Regeneration"*** being submitted by **Mr. Nilotpal Majumder** to the **Indian Institute of Technology Delhi** for the award of the degree of **"DOCTOR OF PHILOSOPHY",** is a record of the original research work carried out by him under my supervision and guidance. He has fulfilled all the requirements for submitting this thesis, which, to the best of my knowledge, meets the required standard.

The material in this thesis has not been submitted in part or in full to any other University or Institute for the award of any other degree.

Prof. Sourabh Ghosh

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Abstract

The progressive degradation of articular cartilage is a significant hallmark in the onset of osteoarthritis (OA). Aging, traumatic injury, excessive physical exercise, obesity, hyperlipidaemia, elevation in blood sugar level, as well as estrogen deficiency (menopausal women) and synovial inflammation are considered key risk factors that accelerate the degeneration of healthy, functioning articular cartilage. Clinicians worldwide are mainly focused on pain management therapy by prescribing various non-steroidal anti-inflammatory drugs (NSAIDs), visco-supplementation, steroidal injections and physiotherapy as a few available non-surgical therapeutic approaches to contain the symptoms of OA. The surgical treatment strategy in such cases (Total knee arthroplasty or Unicompartmental knee arthroplasty) further aggravates the patient's condition, leading to additional problems of post-operative pain, joint stiffness, and *in situ* infections. This compelled the researchers to adopt tissue-engineering-based strategies using a diverse combination of biomaterials, cell types, and scaffolding techniques to regenerate damaged cartilage in an osteoarthritic environment, aiming for long-term symptomatic relief in OA. However, only a few approaches could successfully overcome the benchmarks of clinical trials. This is because the majority of strategies focus on the traditional chondrogenic differentiation protocol, which uses exogenous growth factors and short-lived small-molecule supplements, often in undesired combinations of complex biomaterials. This can be further attributed to the absence of immunomodulation, reactive oxygen species clearance, hypersecretion of mechanically inferior collagen type I matrices, or the formation of transient cartilage rather than hyaline cartilage in an OA microenvironment. Therefore, the present study utilizes chemically modified silk fibroin in various formats, ranging from protective nanocoatings to injectable hydrogels, ultimately leading to 3D bioprinted synthetic cartilage tissue mimics that can successfully regenerate neo-articular cartilage while inhibiting hypertrophic differentiation within a full-thickness cartilage defect in an OA rat model.

A silk protein ionomer-based layer-by-layer (LBL) nanocoating strategy was utilized to preserve the viability, metabolic activity, and differentiation potential of human mesenchymal stem cells (hMSCs) in an *in vitro* inflamed osteoarthritis (OA) tissue niche. These temporary ionomer coatings for cell protection were functionalized with cytokines to impart immune-modulatory behavior. The cytokine-functionalized ionomer nanocoatings polarized neutral M0 macrophages into M1 and M2 subtypes, depending on the specific cytokine used, thereby

reducing synovial inflammation in an *in vitro* setup. This approach would potentially abrogate the need for *ex vivo* polarization before transplantation during immunotherapy. The modular nature of these silk ionomer nanocoatings for tailoring cell surface stability was further validated by extending the cell coatings to 3D spheroids and by introducing tyramine into the cationic silk ionomer to facilitate covalent crosslinking in addition to electrostatic interactions between the cell surface and the ionomers. These results demonstrate the versatile and modular nature of silk-based ionomer cell nanocoatings, allowing control at material, cellular, and spheroid levels to tune coating stability and inflammatory responses. These systems uniquely impart immunomodulatory function, providing a biomaterial platform for cell-based immune-regenerative therapy, such as for OA and other inflammatory diseases.

The existing cartilage tissue engineering strategies fail to recapitulate the native cartilage extracellular matrix (ECM) cues in their scaffolds, which not only exhibit pro-chondrogenic traits but also exert chondroprotective effects against the surrounding harsh osteoarthritic (OA) niche. As a result, the cartilage tissue that forms undergoes fibrosis, which often goes unnoticed and remains uncharacterized. Thus, in our current study, we designed glucosamine (GlcN)-functionalized silk fibroin (SF) with varying degrees of substitution, leveraging different reactive amino acid side chains (SF(S)-GlcN, SF(D, E)-GlcN), in combination with tyramine-modified gelatin (G-TA), to simulate the *in vivo* hyaline cartilage ECM composition. The resultant injectable hydrogel matrix, in the presence of horseradish peroxidase and hydrogen peroxide, can perform a multitude of functions, including antioxidant and anti-inflammatory activities, chondrogenesis, and anti-fibrotic roles. Physicochemical and rheological analysis of the hydrogels demonstrated structural stability, viscoelastic behavior, and modulus recovery, key parameters for an injectable hydrogel. The microporous hydrogel networks supported the migration, adhesion, and proliferation of human mesenchymal stem cells (hMSCs) at passage 2 for both SF(S)-GlcN/G-TA and SF(D, E)-GlcN/G-TA hydrogels. Extensive biological characterization revealed that SF(S)-GlcN/G-TA has better antioxidant, anti-inflammatory, pro-chondrogenic, and anti-fibrotic traits, necessitating a dependency on the glucosamine substitution (high for SF(S)-GlcN) for the chondroprotective effects of the hydrogel systems. Therefore, the present study demonstrates a multifunctional cartilage ECM-mimicking hydrogel system with clinical potential for neo-cartilage regeneration in an OA microenvironment. The transforming growth factor β (TGF-β) cytokine family plays a significant role in articular cartilage formation, from mesenchymal condensation to chondrogenic differentiation. However, the exogenous addition of these factors to the

chondrogenic media or their immobilization onto the scaffolds makes the protocol expensive. Additionally, it reduces the cytokine's bioavailability to cells due to its burst release. Our study demonstrates an advanced bioconjugation strategy for covalently linking TGFβ3 to a silk fibroin matrix via cyanuric chloride coupling. The tethering and change in secondary conformation were confirmed using various spectroscopic analyses. To assess the biological functionality of the chemically modified silk matrix, human BMSCs and chondrocytes were cultured for 28 days in a chondrogenic differentiation medium. Gene expression and histological analysis revealed enhanced expression of chondrogenic markers, accompanied by intense Safranin-O and Alcian Blue staining, in TGFβ3-conjugated silk matrices compared with exogenously added TGFβ3 in the media for both human BMSCs and chondrocytes. Therefore, this study successfully recapitulated the native niche of TGFβ3 and the role of the silk matrix as a stabilizer of growth factors. When cultured on TGFβ3-conjugated silk matrices, human BMSCs exhibited increased proteoglycan secretion and displayed a maximum chondrogenic trait, with attenuation of chondrocyte hypertrophy compared to human chondrocytes.

Implantation of a phenotypically stable cartilage graft could represent a viable approach for repairing osteoarthritic (OA) cartilage lesions. In the present study, we investigated the effects of modulating the Bone Morphogenetic Protein (BMP), Transforming Growth Factor beta (TGFβ), and Interleukin-1 (IL-1) signaling cascades in human bone marrow stromal cells (hBMSCs) encapsulated in silk fibroin-gelatin (SF-G) bioink. The selected small molecules, LDN193189, TGFβ3, and IL-1 receptor antagonist (IL1Ra), are covalently conjugated to SF-G biomaterial to ensure sustained release, increased bioavailability, and printability, as confirmed by ATR-FTIR, release kinetics, and rheological analyses. The 3D bioprinted constructs with chondrogenically differentiated hBMSCs were incubated in an *in vitro* OA-inducing medium for 14 days and assessed using detailed qPCR, Immunofluorescence, and biochemical analyses. Despite substantial heterogeneity in the observations among donors, the IL1Ra molecule demonstrated the greatest efficiency in enhancing the expression of articular cartilage components, reducing the expression of hypertrophic markers (as validated by the GeneMANIA tool), and reducing the production of inflammatory molecules by hBMSCs. Therefore, this study demonstrates a novel strategy for developing a chemically decorated, printable biomimetic SF-G bioink to produce hyaline cartilage grafts resistant to the development of OA traits, which can be used to treat degenerated cartilage lesions.

Regenerating articular cartilage within osteoarthritis (OA)-affected joints remains challenging due to an incomplete understanding of cartilage development and repair. Existing tissue engineering strategies often yield suboptimal hypertrophic or fibrotic cartilage. This study introduces a novel chemically modified silk-based biomaterial that modulates key signaling pathways involved in OA and cartilage repair by concurrently inhibiting the pro-osteogenic bone morphogenetic protein (BMP) pathway and inherently activating the Wnt/β-catenin signaling pathway, a crucial pathway for chondrogenic differentiation and cartilage homeostasis. To achieve this dual modulation, the potent selective BMP inhibitor LDN-193189 was covalently conjugated to Silk Fibroin–Gelatin (SF-G) bioink using cyanuric chloride. Silk, with its inherent ability to activate the pro-chondrogenic Wnt pathway, served as a biomimetic delivery platform, providing robust mechanical resilience and thus supporting the regeneration of load-bearing articular cartilage. The 3D bioprinted LDN-193189-conjugated SF-G construct, encapsulated with rat BMSCs, demonstrated enhanced *in vitro* chondrogenesis, anti-hypertrophic characteristics, and optimal biodegradability. The functionalized biomaterial was implanted into an overcritical-sized cartilage defect in a rat's femoral trochlear groove, which had already been subjected to anterior cruciate ligament transection (ACLT)-induced osteoarthritis. *In vivo* studies confirmed successful neocartilage regeneration, as evidenced by dense Safranin O staining and Collagen Type II expression. The absence of hypertrophic, fibrocartilage, and inflammatory markers indicates the authenticity of the newly formed hyaline cartilage, even in a severe osteoarthritic environment. Overall, the LDN-193189-conjugated silk-based material overcame the challenges of maintaining the hyaline nature of neocartilage regenerated in osteoarthritic joints, indicating a promising regenerative therapeutic approach for OA.

सार

संधि उपास्थि का क्रमिक क्षरण ऑस्टियोआर्थराइटिस (OA) की शुरुआत का एक महत्वपूर्ण लक्षण है। उम्र बढ़ना, आघातजन्य चोट, अत्यधिक शारीरिक व्यायाम, मोटापा, हाइपरलिपिडिमिया, रक्त शर्करा के स्तर में वृद्धि, साथ ही एस्ट्रोजन की कमी (रजोनिवृत्त महिलाओं में) और सिनोवियल सूजन, स्वस्थ, कार्यशील संधि उपास्थि के क्षरण को तेज करने वाले प्रमुख जोखिम कारक माने जाते हैं। दुनिया भर के चिकित्सक मुख्य रूप से दर्द प्रबंधन चिकित्सा पर ध्यान केंद्रित कर रहे हैं, जिसमें OA के लक्षणों को नियंत्रित करने के लिए विभिन्न गैर-स्टेरायडल सूजनरोधी दवाएं (NSAIDs), विस्को-सप्लीमेंटेशन, स्टेरायडल इंजेक्शन और फिजियोथेरेपी जैसे कुछ उपलब्ध गैर-शल्य चिकित्सा उपचार शामिल हैं। ऐसे मामलों में शल्य चिकित्सा उपचार रणनीति (टोटल नी आर्थ्रोप्लास्टी या यूनिकंपार्टमेंटल नी आर्थ्रोप्लास्टी) रोगी की स्थिति को और बिगाड़ देती है, जिससे ऑपरेशन के बाद दर्द, जोड़ों में अकड़न और संक्रमण जैसी अतिरिक्त समस्याएं पैदा हो जाती हैं। इसने शोधकर्ताओं को ऑस्टियोआर्थ्रिटिक वातावरण में क्षतिग्रस्त उपास्थि को पुनर्जीवित करने के लिए बायोमटेरियल, सेल प्रकार और मचान तकनीकों के एक ज्वलंत संयोजन का उपयोग करके ऊतक इंजीनियरिंग-आधारित रणनीतियों को अपनाने के लिए मजबूर किया, जिसका लक्ष्य ओए में दीर्घकालिक लक्षणात्मक राहत प्राप्त करना था। हालांकि, केवल कुछ दृष्टिकोण ही नैदानिक परीक्षणों के मानदंडों को सफलतापूर्वक पार कर सके। ऐसा इसलिए है क्योंकि अधिकांश रणनीतियाँ पारंपरिक चोंड्रोजेनिक भेदभाव प्रोटोकॉल पर अधिक ध्यान केंद्रित करती हैं, जो बहिर्जात वृद्धि कारकों और छोटे-अणु की खुराक का उपयोग बहुत कम अर्ध-आयु के साथ करती है, अक्सर अवांछित जटिल बायोमटेरियल संयोजनों के साथ। इसे इम्यूनोमॉड्यूलेशन की अनुपस्थिति, प्रतिक्रियाशील ऑक्सीजन प्रजातियों की निकासी, यांत्रिक रूप से निम्न कोलेजन प्रकार I मैट्रिसेस के हाइपरसेक्रेशन, या ओए माइक्रोएन्वायरमेंट के संपर्क में आने पर हाइलिन उपास्थि के बजाय क्षणिक उपास्थि के गठन के लिए जिम्मेदार ठहराया जा सकता है। इसलिए, वर्तमान अध्ययन में विभिन्न स्वरूपों में रासायनिक रूप से संशोधित रेशम का उपयोग किया गया है, जिसमें सुरक्षात्मक नैनोकोटिंग से लेकर इंजेक्टेबल हाइड्रोजेल तक शामिल हैं, जो अंततः 3D बायोप्रिंटेड सिंथेटिक उपास्थि ऊतक की नकल करता है जो एक ओए चूहे मॉडल में पूर्ण-मोटाई उपास्थि दोष के भीतर हाइपरट्रॉफिक भेदभाव को रोकते हुए नव-आर्टिकुलर उपास्थि को सफलतापूर्वक पुनर्जीवित कर सकता है।

रेशम प्रोटीन आयनोमर-आधारित परत-दर-परत (एलबीएल) नैनोकोटिंग रणनीति का उपयोग मानव मेसेनकाइमल स्टेम कोशिकाओं (एचएमएससी) की व्यवहार्यता, उपापचयी क्रियाशीलता और विभेदन

क्षमता को इन-विट्रो सूजन वाले ऑस्टियोआर्थराइटिस (ओए) ऊतक निके में संरक्षित करने के लिए किया गया था। कोशिका सुरक्षा के लिए इन अस्थायी आयनोमर कोटिंग्स को प्रतिरक्षा-संशोधक व्यवहार प्रदान करने के लिए साइटोकाइन्स के साथ क्रियाशील बनाया गया था। साइटोकाइन-क्रियाशील आयनोमर नैनोकोटिंग्स ने तटस्थ M0 मैक्रोफेज को M1 और M2 उपप्रकारों में ध्रुवीकृत किया, जो प्रयुक्त विशिष्ट साइटोकाइन पर निर्भर करता है, जिससे इन-विट्रो सेटअप में सिनोवियल सूजन कम हो जाती है। यह दृष्टिकोण संभावित रूप से प्रतिरक्षा चिकित्सा के दौरान प्रत्यारोपण से पहले एक्स विवो ध्रुवीकरण की आवश्यकता को समाप्त कर देगा। कोशिका सतह स्थिरता को अनुकूलित करने में इन रेशम आयनोम नैनोकोटिंग्स की मॉड्यूलर प्रकृति को कोशिका कोटिंग्स को 3D गोलाकार तक विस्तारित करके और कोशिका सतह और आयनोमर्स के बीच इलेक्ट्रोस्टैटिक अंतःक्रियाओं के अलावा सहसंयोजक क्रॉसलिंकिंग को सुगम बनाने के लिए धनायनिक रेशम आयनोमर्स में टायरामाइन डालकर और भी पुष्ट किया गया। ये परिणाम रेशम-आधारित आयनोमर्स कोशिका नैनोकोटिंग्स की बहुमुखी और मॉड्यूलर प्रकृति को प्रदर्शित करते हैं, जो कोटिंग स्थिरता और सूजन प्रतिक्रियाओं को समायोजित करने के लिए पदार्थ, कोशिकीय और गोलाकार स्तरों पर नियंत्रण की अनुमति देते हैं। ये प्रणालियाँ विशिष्ट रूप से प्रतिरक्षा-नियंत्रक कार्य प्रदान करती हैं, जो कोशिका-आधारित प्रतिरक्षा-पुनर्योजी चिकित्सा, जैसे कि ओए और अन्य सूजन संबंधी रोगों के लिए, एक जैव-पदार्थ मंच प्रदान करती हैं।

मौजूदा उपास्थि ऊतक इंजीनियरिंग रणनीतियाँ अपने स्कैफोल्ड में मूल उपास्थि बाह्य कोशिकीय मैट्रिक्स (ईसीएम) संकेतों को दोहराने में विफल रहती हैं, जो न केवल प्रो-कॉन्ड्रोजनिक लक्षण प्रदर्शित करती हैं बल्कि आसपास के कठोर ऑस्टियोआर्थ्रिटिक (ओए) आला के खिलाफ कॉन्ड्रोप्रोटेक्टिव उत्तेजना भी डालती हैं। नतीजतन, बनने वाले उपास्थि ऊतक फाइब्रोसिस से गुजरते हैं, जो अक्सर अनदेखा और अज्ञात रहता है। इस प्रकार, हमारे वर्तमान अध्ययन में, हमने एक ग्लूकोसामाइन-फंक्शनलाइज्ड सिल्क फाइब्रोइन (दो प्रतिस्थापन मार्ग: सेरीन (एस) और ग्लूटामेट, एस्पार्टेट (डी, ई)) जिलेटिन इंजेक्टेबल हाइड्रोजेल सिस्टम तैयार किया है जो एंटीऑक्सीडेंट और एंटी-इंफ्लेमेटरी गतिविधियों से लेकर कॉन्ड्रोजनिक भेदभाव और फाइब्रोसिस अवरोध तक कई कार्यों को कर सकता है। मैक्रोपोरस हाइड्रोजेल नेटवर्क ने SF(S)-GlcN-G-TA और SF(D,E)-GlcN-G-TA हाइड्रोजेल दोनों के लिए मानव मेसेनकाइमल स्टेम कोशिकाओं (hMSCs) के प्रवास, आसंजन और प्रसार को बढ़ावा दिया। व्यापक जैविक लक्षण-निर्धारण से पता चला कि SF(S)-GlcN-G-TA रेडॉक्स, इम्यूनोमॉड्यूलेटरी और आर्टिकुलर-कार्टिलेज विभेदन गुणों को प्रदर्शित करने में बेहतर है, जिससे हाइड्रोजेल प्रणालियों के उपास्थि-सुरक्षात्मक प्रभावों के लिए ग्लूकोसामाइन प्रतिस्थापन (SF(S)-GlcN के लिए उच्च) पर निर्भरता आवश्यक हो गई। इसलिए, यह अध्ययन एक बहुक्रियाशील कार्टिलेज ECM-अनुकरण करने वाली

हाइड्रोजेल प्रणाली को प्रदर्शित करता है जिसमें OA सूक्ष्म वातावरण में नव-कार्टिलेज पुनर्जनन की नैदानिक क्षमता है।

साइटोकाइन्स का रूपांतरणकारी वृद्धि कारक β वर्ग, मेसेंकाइमल संघनन से लेकर उपास्थि निर्माण में उपास्थिजन्य विभेदन तक महत्वपूर्ण भूमिका निभाता है। हालाँकि, उपास्थिजन्य माध्यम में इन कारकों का बहिर्जात योग या स्कैफोल्ड पर उनका स्थिरीकरण इस प्रक्रिया को महंगा बना देता है। इसके अतिरिक्त, यह कोशिकाओं में साइटोकाइन की जैवउपलब्धता को उनके फटने के कारण कम कर देता है। हमारा अध्ययन एक सायन्यूरिक क्लोराइड युग्मन अभिक्रिया के माध्यम से TGFβ3 को रेशम फ़ाइब्राइन मैट्रिक्स के साथ सहसंयोजक रूप से संयुग्मित करने की एक उन्नत जैवसंयुग्मन रणनीति को प्रदर्शित करता है। विभिन्न स्पेक्ट्रोस्कोपिक विश्लेषणों का उपयोग करके टेदरिंग और द्वितीयक संरूपण में परिवर्तन की पुष्टि की गई। रासायनिक रूप से संशोधित रेशम मैट्रिक्स की जैविक कार्यक्षमता का आकलन करने के लिए, मानव BMSCs और उपास्थिजन्य कोशिकाओं को उपास्थिजन्य विभेदन माध्यम में 28 दिनों तक संवर्धित किया गया। जीन अभिव्यक्ति और ऊतकवैज्ञानिक विश्लेषण से पता चला कि TGFβ3-संयुग्मित रेशम आव्यूहों में, तीव्र सफ्रानिन-O और एल्शियन ब्लू अभिरंजन के साथ, चोंड्रोजेनिक मार्करों की अभिव्यक्ति में वृद्धि हुई, जबकि TGFβ3 को मानव BMSCs और उपास्थि कोशिकाओं, दोनों के लिए माध्यम में बाह्य रूप से मिलाए जाने पर यह वृद्धि देखी गई। इसलिए, इस अध्ययन ने TGFβ3 के मूल निकेत और वृद्धि कारकों के स्थिरक के रूप में रेशम आव्यूह की भूमिका को सफलतापूर्वक दोहराया। TGFβ3-संयुग्मित रेशम आव्यूहों पर संवर्धित होने पर, मानव BMSCs ने प्रोटियोग्लाइकन स्राव में वृद्धि और अधिकतम उपास्थि कोशिकाओं की विशेषता प्रदर्शित की, साथ ही मानव उपास्थि कोशिकाओं की तुलना में उपास्थि कोशिका अतिवृद्धि में कमी देखी गई।

फेनोटाइपिक रूप से स्थिर कार्टिलेज ग्राफ्ट का प्रत्यारोपण ऑस्टियोआर्थ्रिटिक (OA) कार्टिलेज घावों की मरम्मत के लिए एक व्यवहार्य दृष्टिकोण प्रस्तुत कर सकता है। वर्तमान अध्ययन में, हमने सिल्क फ़ाइब्रोइन-जिलेटिन (SF-G) बायोइंक में संपुटित मानव अस्थि मज्जा स्ट्रोमल कोशिकाओं (hBMSCs) में बोन मॉर्फोजेनेटिक प्रोटीन (BMP), ट्रांसफ़ॉर्मिंग ग्रोथ फ़ैक्टर बीटा (TGFβ), और इंटरल्यूकिन-1 (IL-1) सिग्नलिंग कैस्केड के मॉड्यूलेशन के प्रभावों की जाँच की। चयनित छोटे अणु, LDN193189, TGFβ3, और IL-1 रिसेप्टर प्रतिपक्षी (IL1Ra), SF-G बायोमटेरियल से सहसंयोजक रूप से संयुग्मित होते हैं ताकि निरंतर रिलीज़, बढ़ी हुई जैवउपलब्धता और प्रिंटेबिलिटी सुनिश्चित हो सके, जैसा कि ATR-FTIR, रिलीज़ कैनेटीक्स और रियोलॉजिकल विश्लेषणों द्वारा पुष्टि की गई है। उपास्थिजनित रूप से विभेदित hBMSCs युक्त त्रि-आयामी जैव-मुद्रित संरचनाओं को इन विट्रो OA-प्रेरक माध्यम में 14 दिनों तक संवर्धित किया

गया और विस्तृत qPCR, इम्यूनोफ्लोरेसेंस और जैव-रासायनिक विश्लेषणों के माध्यम से उनका मूल्यांकन किया गया। दाताओं के बीच अवलोकनों में पर्याप्त विविधता के बावजूद, IL1Ra अणु ने संधि उपास्थि घटकों की अभिव्यक्ति को बढ़ाने, हाइपरट्रॉफिक मार्करों की अभिव्यक्ति को कम करने (जैसा कि GeneMANIA उपकरण द्वारा प्रमाणित है), और hBMSCs द्वारा भड़काऊ अणुओं के उत्पादन को कम करने में अधिकतम दक्षता प्रदर्शित की। इसलिए, यह अध्ययन OA लक्षणों के विकास के प्रति प्रतिरोधी हाइलाइन उपास्थि ग्राफ्ट का उत्पादन करने हेतु एक रासायनिक रूप से अलंकृत, मुद्रण योग्य बायोमिमेटिक SF-G बायोइंक विकसित करने की एक नवीन रणनीति प्रदर्शित करता है, जिसका उपयोग विकृत उपास्थि घावों के उपचार के लिए किया जा सकता है।

ऑस्टियोआर्थराइटिस (OA) से प्रभावित जोड़ों में आर्टिकुलर कार्टिलेज का पुनर्जनन, कार्टिलेज के विकास और मरम्मत की अपूर्ण समझ के कारण, चुनौतीपूर्ण बना हुआ है। मौजूदा ऊतक अभियांत्रिकी रणनीतियाँ अक्सर निम्न-इष्टतम हाइपरट्रॉफिक या फाइब्रोटिक कार्टिलेज उत्पन्न करती हैं। यह अध्ययन एक नवीन रासायनिक रूप से संशोधित रेशम-आधारित जैव-सामग्री प्रस्तुत करता है जो प्रो-ऑस्टियोजेनिक बोन मॉर्फोजेनेटिक प्रोटीन (BMP) मार्ग को एक साथ बाधित करके और Wnt/β-कैटेनिन सिग्नलिंग मार्ग, जो कि उपास्थिजन्य विभेदन और उपास्थि होमियोस्टेसिस के लिए एक महत्वपूर्ण मार्ग है, को स्वाभाविक रूप से सक्रिय करके OA और उपास्थि की मरम्मत में शामिल प्रमुख संकेतन मार्गों को नियंत्रित करता है। इस दोहरे नियंत्रण को प्राप्त करने के लिए, शक्तिशाली चयनात्मक BMP अवरोधक LDN-193189 को सायन्यूरिक क्लोराइड का उपयोग करके सिल्क फाइब्रोइन-जिलेटिन (SF-G) बायोइंक के साथ सहसंयोजक रूप से संयुग्मित किया गया। प्रो-कॉन्ड्रोजेनिक Wnt मार्ग को सक्रिय करने की अपनी अंतर्निहित विशेषताओं के साथ, सिल्क ने एक बायोमिमेटिक वितरण प्लेटफ़ॉर्म के रूप में कार्य किया, जो मज़बूत यांत्रिक लचीलापन प्रदान करता है और इस प्रकार भार वहन करने वाले आर्टिकुलर कार्टिलेज के पुनर्जनन में सहायक होता है। चूहे के BMSCs से युक्त, 3D बायोप्रिंटेड LDN-193189-संयुग्मित SF-G संरचना ने इन विट्रो कॉन्ड्रोजेनिक गुणों में वृद्धि, एंटी-हाइपरट्रॉफिक विशेषताओं और इष्टतम जैवनिम्नीकरण क्षमता का प्रदर्शन किया। क्रियाशील बायोमटेरियल को चूहे के फीमरल ट्रोक्लियर ग्रूव में एक अति-महत्वपूर्ण आकार के कार्टिलेज दोष में प्रत्यारोपित किया गया, जो पहले से ही एंटीरियर क्रूसिएट लिगामेंट ट्रांसेक्शन (ACLT)-प्रेरित ऑस्टियोआर्थराइटिस से ग्रस्त था। इन विवो अध्ययनों ने सघन सैफ्रानिन O अभिरंजन और कोलेजन प्रकार II की अभिव्यक्ति के माध्यम से सफल नियोकार्टिलेज पुनर्जनन की पुष्टि की। हाइपरट्रॉफिक, फाइब्रोकार्टिलेज और इन्फ्लेमेटरी मार्करों की अनुपस्थिति, गंभीर

ऑस्टियोआर्थ्रिटिक वातावरण में भी, नवनिर्मित हाइलाइन कार्टिलेज की प्रामाणिकता को इंगित करती है। कुल मिलाकर, LDN-193189-संयुग्मित रेशम-आधारित पदार्थ ने ऑस्टियोआर्थ्रिटिक जोड़ों में पुनर्जीवित नियोकार्टिलेज की हाइलाइन प्रकृति को बनाए रखने की चुनौतियों पर विजय प्राप्त की, जो ऑस्टियोआर्थ्रिटिक जोड़ों के लिए एक आशाजनक पुनर्योजी चिकित्सीय दृष्टिकोण का संकेत देता है।

Table of Contents

Serial no.	Content	Page number
	Acknowledgements	i
	Abstract	iii
	List of Figures	xix
	List of Tables	xxx
1.	**Introduction**	1
1.1	Introduction to articular cartilage degeneration	2
1.2	The molecular and cellular level differences in permanent articular cartilage and transient cartilage	3
1.2.1	Articular cartilage development	3
1.2.2	Transient cartilage development	4
1.2.3	Common point of origin for permanent and transient cartilage	4
1.3	Tissue engineering approaches for articular cartilage regeneration and OA management	5
1.3.1	Single-cell transplantation	5
1.3.2	3D aggregate	6
1.3.3	3D Bioprinted models	7
1.4	The major reason behind the futile cartilage tissue engineering strategies: Chondrocyte hypertrophy	11
1.4.1	Introduction to hypertrophic chondrocytes	11
1.4.2	Onset of chondrocyte hypertrophy during *in vitro* chondrogenic differentiation	12
1.5	Other existing challenges of cartilage tissue engineering	19
1.5.1	Cellular Senescence	19
1.5.2	Chondrocyte dedifferentiation and fibrosis	20
1.5.3	Lack of efficient molecular characterization	21
1.6	Scope of the present study	23
1.7	Research objectives	24
2	**Silk Ionomer-based Modular Nanocoatings for Potential Immuno-regenerative Cell Therapy in Osteoarthritis**	25
2.1	Introduction	26
2.2	Materials and Methods	28
2.2.1	Preparation of positively charged and negatively charged ionomer solutions	28

2.2.2	Procedure of nanoencapsulation on the cell surface	28
2.2.3	Evaluating the stability and physicochemical properties of nanocoating	29
2.2.3.1	Fluorescence microscopy	29
2.2.3.2	Scanning Electron Microscopy (SEM)	29
2.2.3.3	Atomic Force Microscopy (AFM)	29
2.2.4	Evaluating the cytocompatibility and immunocompatibility of the nanocoatings	30
2.2.4.1	Cytocompatibility	30
2.2.4.2	Differentiation potential of the hMSCs after nano-encapsulation	30
2.2.4.3	Evaluating the fate of the nanocoatings in the body	31
2.2.5	Evaluating the protective nature of the nanocoatings	31
2.2.5.1	Simulated joint microenvironmental stress conditions	31
2.2.5.2	Simulated joint mechanical stress	32
2.2.6	Developing a cytokine-immobilized nanocoating for immunomodulation	32
2.2.6.1	Anti-inflammatory coated human chondrocytes promote re-polarization of pro-inflammatory M1 macrophages to a healing M2 phenotype	32
2.2.6.2	Coating-directed polarization of M0 macrophages polarization	33
2.2.6.3	Coating-directed polarization of THP-1 ASC-derived macrophages	33
2.2.6.4	Immunomodulatory effect of the anti-inflammatory coated M0 macrophages on the inflammatory synovial fibroblasts	34
2.2.7	Effect of nanocoatings on 3D cell aggregates	34
2.2.7.1	Nanoencapsulation of spheroids	34
2.2.7.2	Evaluating the coating stability and viability of the spheroids post-encapsulation	35
2.2.7.3	Evaluating the protective nature of the nanocoatings from microenvironmental stress and inflammatory immune cell attack	35
2.2.8	Developing a tunable tyramine-substituted ionomer (SF-EDA-TA) for enhanced stability	36
2.2.8.1	Ionomer synthesis	36
2.2.8.2	Characterization of the SF-EDA-TA ionomer	36
2.2.8.3	Stability and cytocompatibility of the SF-EDA-TA nanocoatings on stem cells and immune cells	37
2.2.8.4	Stability of the SF-EDA-TA coating in 3D aggregates	37
2.2.9	Statistical analysis	38
2.3	Results and Discussion	38
2.3.1	Silk ionomer nanocoatings did not alter hMSCs' functions	38

2.3.2	Ionomer shells shielded hMSCs against catabolic and biomechanical stressors of the knee joint	42
2.3.2.1	Catabolic stress	42
2.3.2.2	Biomechanical stress	45
2.3.3	Degradation fate mapping of nanocoatings	45
2.3.4	Immunomodulatory, anti-inflammatory nanocoating inhibited hypertrophic differentiation of the chondrocytes and promoted M1 macrophage repolarization	50
2.3.5	Macrophage polarization driven by nanoencapsulation inhibited synovial fibroblast inflammation	55
2.3.6	3D spheroids exhibited superior nanocoating retention than single cells with effective immune evasion	60
2.3.7	Co-functionalization of EDA and TA onto SF strengthened the structural stability of the nanocoatings through additional di-tyrosine linkage	64
2.4	Conclusion	68
3	**Glucosamine-functionalised silk gelatin composite hydrogel to support chondrogenic differentiation of stem cells**	71
3.1	Introduction	72
3.2	Materials and Methods	76
3.2.1	Preparation of sugar-functionalized silk	76
3.2.1.1	Preparation of aqueous silk solution	76
3.2.1.2	Serine carboxylation of regenerated silk fibroin (SF(S)-COOH)	76
3.2.1.3	Glucosamine modification onto carboxylated silks (SF(S)-GlcN)	76
3.2.1.4	Glucosamine functionalization onto regenerated silk fibroin (SF (D, E)-GlcN)	77
3.2.2	Synthesis of tyramine-modified gelatin (G-TA)	77
3.2.3	Hydrogel preparation	78
3.2.4	Physicochemical characterization of the composite hydrogel	78
3.2.4.1	Fourier transform infrared (FT-IR) spectroscopy	78
3.2.4.2	Rheological characterization	78
3.2.4.3	Scanning electron microscopy (SEM)	79
3.2.5	Biological characterization	79
3.2.5.1	hMSC expansion	79
3.2.5.2	Evaluation of cell adherence and morphology	79
3.2.5.3	Evaluation of cytocompatibility	80
3.2.5.4	Evaluation of Immunocompatibility	80
3.2.5.5	Evaluation of antioxidant properties	81

3.2.5.6	Evaluation of chondrogenic differentiation	81
3.3	Results	82
3.3.1	Molecular weight estimation	82
3.3.2	Cytocompatibility of the glucosamine-modified silks	82
3.3.3	Structural stability analysis of the glucosamine-modified silk gelatin composite hydrogels	85
3.3.4	Rheological profile of the hydrogel	87
3.3.5	Surface morphology of the hydrogels	89
3.3.6	hMSC cell morphology and spreading on the hydrogels	92
3.3.7	Cell viability and proliferation on the SF-GlcN matrices and composite hydrogels	92
3.3.8	Innate immune response of the composite hydrogels	93
3.3.9	ROS scavenging characteristic of the composite hydrogels	93
3.3.10	Chondrogenic differentiation ability of the composite hydrogels	97
3.4	Discussion	102
3.5	Conclusion	106
4	**An advanced bioconjugation strategy to simulate the ECM-embedded latent TGFβ3 system within the silk fibroin matrix and its implication in chondrogenesis**	107
4.1	Introduction	108
4.2	Materials and Methods	111
4.2.1	Molecular Modelling	111
4.2.2	Preparation of SF solution, films, and hydrogel	112
4.2.3	Reaction of SF with CyCl and TGFβ3	112
4.2.4	Fourier transform infrared spectroscopy (FTIR) analysis	113
4.2.5	Nuclear Magnetic Resonance (NMR) analysis	113
4.2.6	Circular Dichroism (CD) analysis	113
4.2.7	Isolation and Expansion of Human Articular Chondrocytes (hACs)	114
4.2.8	Human bone marrow-derived mesenchymal stem cells (hBMSCs) expansion	114
4.2.9	Cell seeding on SF matrices	114
4.2.10	Gene expression analysis	115
4.2.11	Scanning electron microscopy (SEM)	116
4.2.12	Glycosaminoglycan (GAG) estimation	116
4.2.13	Histology	116
4.2.14	Statistical analysis	117

4.3	Results and Discussion	117
4.3.1	Molecular Modelling: Structure & Interaction	117
4.3.2	FTIR analysis	118
4.3.3	NMR analysis	124
4.3.4	CD analysis	126
4.3.5	Cellular morphology analysis	127
4.3.6	GAG estimation	128
4.3.7	Gene expression analysis	129
4.3.8	Histological analysis	135
4.4	Conclusion	137
5	**Covalent conjugation of small molecule inhibitors and growth factors to a silk fibroin-derived bioink to develop phenotypically stable 3D bioprinted cartilage *in vitro***	138
5.1	Introduction	139
5.2	Materials and Methods	141
5.2.1	Computational modeling of bioconjugation	141
5.2.2	Preparation of silk fibroin solution	142
5.2.3	Chemical modification of SF biomaterial	142
5.2.3.1	Cyanuric chloride-mediated coupling of LDN193189 and TGFβ3	142
5.2.3.2	Diazonium coupling-mediated crosslinking of IL1Ra	142
5.2.4	Physicochemical characterization	143
5.2.4.1	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)	143
5.2.4.2	UV-visible spectroscopy	143
5.2.5	*In vitro* release kinetics of LDN19389, TGFβ3 and IL1Ra	143
5.2.6	Preparation of silk fibroin gelatin (SF-G) bioink	144
5.2.7	Rheological analysis	144
5.2.8	Printability analysis	144
5.2.9	Expansion and chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs)	145
5.2.10	Evaluation of chondrogenesis of hBMSCs	145
5.2.11	3D bioprinting of cell-encapsulated chemically modified SF-G bioink	146
5.2.12	Histological analysis	146
5.2.13	Gene expression analysis	146
5.2.14	Protein expression analysis	147

5.2.15	Biochemical analyses	148
5.2.16	Gene analysis using GeneMANIA	148
5.2.17	Statistical analysis	149
5.3	Results	149
5.3.1	Computational prediction of conjugation of small molecules and growth factors to the SF biomaterial	149
5.3.2	Experimental validation of the chemical modification of silk fibroin biomaterial	151
5.3.2.1	ATR FTIR characterization	151
5.3.2.2	UV visible spectroscopy	153
5.3.3	Rheological characterization	153
5.3.4	*In vitro* release kinetics	156
5.3.5	Visualization of the 3D bioprinted constructs	156
5.3.6	Evaluation of chondrogenesis post 14 days of hBMSC chondrogenic differentiation	157
5.3.7	Gene expression analysis	159
5.3.8	Biochemical analyses	163
5.3.8.1	DMMB assay	164
5.3.8.2	Total nitric oxide content	164
5.3.8.3	Total nitrite content	164
5.3.8.4	Lipid peroxidation profile	165
5.3.9	Protein expression analysis	167
5.3.10	Histological evaluation	169
5.3.11	GeneMANIA analysis	170
5.4	Discussion	173
5.5	Conclusion	177
6	**Chemically modified silk fibroin-gelatin 3D bioprinted constructs for neo-cartilage regeneration in a full-thickness cartilage defect within a rat osteoarthritic knee joint**	178
6.1	Introduction	179
6.2	Materials and Methods	182
6.2.1	Preparation of chemically conjugated SF-G constructs	182
6.2.2	Physicochemical characterization	182
6.2.3	Atomic Force Microscopy (AFM)	183
6.2.4	*In vitro* Biological characterization	183
6.2.4.1	qPCR analysis	183

6.2.4.2	Biochemical estimation	184
6.2.4.3	Immunofluorescence and histology analysis	184
6.2.4.4	Dual Luciferase Reporter Assay (BRITER Assay)	184
6.2.5	*In vivo* Study Design	184
6.2.5.1	Sample size	184
6.2.5.2	Data exclusion	184
6.2.5.3	Experimental Design	185
6.2.5.4	Randomization	185
6.2.5.5	Animal study protocols	185
6.2.5.6	3D volumetric projections of Micro-computed tomography (μCT)	185
6.2.5.7	Anterior Cruciate Ligament Transection	185
6.2.5.8	Creating Full-thickness Cartilage Defect and Constructs Implantation	185
6.2.5.9	Tissue Processing, immunohistochemistry, and classical histology	186
6.2.5.10	Blind Data Scoring	187
6.2.5.11	Statistical analysis	187
6.3	Results	188
6.3.1	Evaluation of physicochemical properties and mechanical resilience of a 3D bioprinted LDN-193189 conjugated SF-G construct	188
6.3.2	The 3D bioprinted chemically-modified SF-G construct promotes *in vitro* chondrogenesis of the encapsulated rat bone marrow-derived mesenchymal stem cells (rat BMSCs)	191
6.3.3	LDN-193189-conjugated SF-G constructs Promote Site-Specific Cartilage Repair and Proteoglycan Restoration in Cartilage Defects within the Rat OA Knee Joint.	192
6.3.4	Inhibition of BMP signaling by LDN-193189-conjugated 3D printed constructs promotes regeneration of hyaline cartilage and suppresses hypertrophy in cartilage defects in the rat OA knee joint	195
6.3.5	LDN-193189-conjugated 3D printed constructs attenuate inflammation to create a pro-regenerative niche in cartilage defects in the rat OA knee joint	198
6.4	Discussion	199
6.5	Conclusion	205
7	**Future Directions**	206
7.1	Future Scope of the Research	207
	References	209
	Curriculum Vitae	241

List of Figures

Chapter 1	
1.1	Histology of a) Healthy cartilage and b) Osteoarthritic cartilage. Dense Safranin O staining in healthy cartilage envisages the presence of enormous glycosaminoglycan content, whereas negligible positive staining is observed in the case of osteoarthritic cartilage samples. The fibrillation of the superficial layer and cellular aggregation clearly distinguish the hypertrophic cartilage from the healthy cartilage, marked by an intact superficial layer and homogenous cellular distribution.
1.2	Graphical illustration demonstrating the single point of origin of permanent and transient cartilage, regulated by a wide array of transcription factors and signaling molecules.
1.3	H&E staining of healthy and hypertrophic cartilage demonstrates increased cell volume and cell clustering in the hypertrophic cartilage. The possible reason is the osmotic influx of water through aquaporin channels, driven by changes in the hypertrophic microenvironment's osmolarity. A pertinent question is whether the increase in chondrocyte volume and clustering arises due to hypertrophic maturation or whether this chondrocyte swelling is associated with abnormal (neo)matrix production and directly/indirectly causes cartilage graft failure. Moreover, when the isolated chondrocytes are expanded *in vitro* in 2D, they lose their round morphology and assume a fibroblastic characteristic marked with an increased expression of COL-I instead of COL-II through a process termed 'cellular dedifferentiation'. When these cells are cultured in 3D systems (embedded in a hydrogel), the round morphology of the chondrocyte is restored with an expression of articular cartilage-specific markers. However, the ambiguity lies in the fact that if the 'cellular de-differentiation' phenomenon can be reversed in 3D culture models, what could be the possible source of chondrocyte hypertrophy when the same chondrocyte-laden tissue constructs are implanted *in vivo*?
1.4.	The dysfunctioning of the pro-chondrogenic signaling pathways and their mechanistic crosstalk ensued from the vast array of chondrogenic media supplements and the surrounding *in vitro* biomimetic microenvironment
Chapter 2	
2.1.	The layer-by-layer deposition of silk ionomer nanocoating preserved the viability, proliferation, metabolic activity and differentiation potential of hMSCs. a) Chemical synthesis of the cationic and anionic silk ionomers through carbodiimide and diazonium chemistries, b) Nanoencapsulation mechanism of layer-by-layer deposition of the cationic and anionic silk ionomers on the cell surface, c) Fluorescence microscopy images demonstrating the stability of the nanocoatings on the cell surface up to 72 h with further aggregation at 168 h (day 7) (Scale bar: 100 μm), d) SEM micrographs illustrating the successful deposition of nanocoating on the cell surface (Scale bar: 2 μm), e) Topographic image of non-coated control (Scale

	bar: 4 μm), 3-bilayer and 6-bilayer (Scale bar: 6 μm) nanocoated MSCs using contact mode of AFM, f) Bar graph comparing the Young's modulus of the 3-bilayer and 6-bilayer nanocoated hMSCs with the non-coated control until day 7(*p < 0.05, **p < 0.01 and ***p < 0.001), g) Live/dead imaging showing the viability of the hMSCs post nano-coating (Scale bar: 100 μm), h) Quantification of cell proliferation after nanoencapsulation at days 1, 3 and 7 using Alamar blue assay (*p < 0.05, **p < 0.01 and ***p < 0.001)(normalized to the non-coated control Alamar blue data), i) Quantification of metabolic activity of the MSCs post 3 and 6-bilayer nanocoating at day 7 (*p < 0.05, **p < 0.01 and ***p < 0.001), and j) Intact differentiation potential of the MSCs post-nanocoating (Scale bar:100 μm).
2.2	The protective role of increasing layer thickness in shielding the coated MSCs against OA microenvironmental and biomechanical stressors, a) Graphical representation of the experimental overview to evaluate the efficacy of nanocoating strategies in protecting the cell against OA stress signals, b) Live/dead micrographs showing the viability of nano-encapsulated MSCs incubated in 72 h cocktail stress media (Combination of 0.05 ng/ml of Interleukin 1 beta (IL-1β), 0.05 ng/ml of TNFα and 0.1 ng/ml of Interleukin 6 (IL-6), 0.5 mM of hydrogen peroxide, 0.05 U of Protease XIV and 0.5 mM of $CoCl_2$) (Scale bar: 100 μm), c) Heat map representing the LDH release profile of the 3 and 6-bilayer coated MSCs post 72 h incubation in OA-mimicking catabolic stress media *(INFL: Inflammation media, OS: Oxidative stress media, PS: Protease stress media, Hyp: Hypoxia stress media)*, d) Heat map representing the Alamar blue intensity profile of the 3 and 6-bilayer coated MSCs post 72 h incubation in OA-mimicking catabolic stress media, e) Live/dead micrographs demonstrating the efficacy of 3 and 6-bilayer coated MSCs in protecting the cells from mechanical stress for 150, 300 and 600 s respectively (Scale bar: 100 μm), f) Quantification of cell viability as a function of cell proliferation after subjecting the nanocoated cells to mechanical stress for 150, 300 and 600 s respectively *(*p < 0.05, **p < 0.01 and ***p < 0.001)* (normalized to the control and nanoencapsulated cells subjected to no shear force), g) Evaluation of cell membrane integrity as a function of LDH release quantification after subjecting the nanocoated cells to mechanical stress for 150, 300 and 600 s respectively *(*p < 0.05, **p < 0.01 and ***p < 0.001),* h) Experimental work flow to predict the fate of the nanocoatings post-disengaging from the cell surface, and i, j and k) demonstrating the internalization of the detached SF ionomer by the macrophages using flow cytometry
2.3	Viability assessment of the nanocoated hMSCs following incubation in different OA microenvironmental stressors**,** a) Inflammation (0.05 ng/ml IL-1β, 0.05 ng/ml of TNFα and 0.1 ng/ml of IL-6), b) 0.1 mM H_2O_2 (Oxidative stress), c) 0.5 mM H_2O_2 (Oxidative stress), d) 0.01 U Protease XIV, e) 0.05 U Protease XIV, f) 0.1 mM $CoCl_2$ (Hypoxia), g) 0.5 mM $CoCl_2$ (Hypoxia); (Scale bar: 100 μm), h) Bar graph representation of the alamar blue assay quantifying the hMSC proliferation rate post-incubation in different OA catabolic stress mediums for 72 h, and i) Bar graph representation of the LDH assay quantifying the hMSC cell membrane integrity post-incubation in

	different OA catabolic stress mediums for 72 h *(*p < 0.05, **p < 0.01 and ***p < 0.001).*
2.4	An anti-inflammatory nanocoating to modulate hypertrophic differentiation of chondrocytes and simultaneously immunomodulate the surrounding inflammatory microenvironment, a) Fluorescence micrographs comparing the expression pattern of hypertrophic marker, collagen type X (COL-X) in the nanocoated chondrocytes post-incubation in hypertrophic medium for 7 days (anti-inflammatory coating: silk ionomer nanocoating functionalized with anti-inflammatory cytokines, standard coating: cationic and anionic silk ionomer, non-coated control: only MSCs without encapsulation) (Scale bar: 100 μm), b) Corresponding relative COL-X fluorescence intensities of the respective non-coated control, standard coating and anti-inflammatory coating experimental groups, c) Graphical representation illustrating the effect of anti-inflammatory nanocoating on the hypertrophic differentiation of chondrocytes, d) Graphical representation of the immunomodulatory effect of the anti-inflammatory nanocoating in promoting M1 repolarization to M2 when co-cultured with anti-inflammatory coated chondrocytes for 72 h, and e) Fluorescence micrographs comparing the potency of M2 phenotype conversion from M1 post-co culturing with anti-inflammatory nanocoated chondrocytes using M1-specific CD86 and M2-specific CD206 marker expressions (Scale bar: 100 μm).
2.5	Quantitative evaluation of the extent of macrophage polarization as a function of fluorescence intensity**,** a, b and c) Relative fluorescence intensity measurements of CD86 and CD206 and M1/M2 ratio assessment, d) Fluorescence intensity calculations of CD86, iNOS, CD206 and Arg staining in nanoencapsulated M1 and M2 macrophages, and e) Fluorescence intensity of α-SMA staining in synoviocytes. All the quantifications were conducted using ImageJ software *(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
2.6	*In situ* nanocoating-mediated polarization of neutral M0 macrophages to M1 or M2 phenotype and their immunoregulatory role in modulating inflamed OA synovium*,* a) Graphical overview of utilizing anti-inflammatory (IL-4 + IL-13+IL1Ra) and pro-inflammatory (LPS + IFNγ) cytokine-functionalized nanocoating in promoting M1 and M2 polarization, b) Fluorescence micrographs confirming the *in situ* differentiation of M0 macrophages to M1 subtype through CD86 and iNOS protein expression (Scale bar: 100 μm) c) Fluorescence micrographs confirming the *in situ* differentiation of M0 macrophages to M2 subtype through CD206 and Arginase protein expression (Scale bar: 100 μm), d) Graphical representation illustrating the differentiation of THP-1 ASC-derived M0 macrophage to M1 phenotype through the pro-inflammatory cytokine-embedded nanocoating, e) Fluorescence micrographs demonstrating green speck formation within the THP-1 ASC derived macrophages upon inflammatory nanocoating (Scale bar: 100 μm), f) Quantification of cleaved IL-1β from the THP-1 ASC-derived M1 macrophages post 72 h inflammatory nanocoating *(*p < 0.05, **p < 0.01 and ***p < 0.001),* g) Graphical representation demonstrating the immunomodulatory effect of anti-inflammatory coated macrophages on the

	surrounding inflamed synovium, h) Fluorescence micrographs depicting the reduction in α-SMA expression after direct co-culturing the anti-inflammatory coated macrophages with the inflamed synovial fibroblasts for 72 h (Scale bar: 100 μm), and i) Quantification of cleaved IL-1β from the inflamed synovial fibroblasts post 72 h co-culture with anti-inflammatory coated macrophages *(*p < 0.05, **p < 0.01 and ***p < 0.001).*
2.7	Increased stability of the nanocoating on the surface of 3D spheroids protected the 3D cellular aggregates from immune surveillance. a) Graphical representation of the deposition of multi-layer cationic and anionic silk ionomers on the surface of 3D spheroids and their subsequent protection of OA microenvironmental stress and attack by the infiltrating M1 macrophages, b) Representative fluorescence micrographs of FITC-labelled nanocoated 20,000 cells/ml cell spheroid at 24 and 72 h time-points respectively (Scale bar: 200 μm), c) Confocal fluorescence micrographs comparing the stability of the nanocoatings on the surface of the 3D spheroids at 24 h and 168 h time points for both 10,000 and 20,000 cells/ml spheroids (Blue: DAPI, red: Phalloidin and green: FITC-labelled silk ionomer coating) (Scale bar: 100 μm), d) A semi-empirical quantification to compare the degradation profile of the nanocoatings between single cell and spheroids by measuring the FITC intensities of the detached FITC-labelled ionomers at days 1, 3 and 7 *(*p < 0.05, **p < 0.01 and ***p < 0.001),* e) Quantification of cell proliferation within the 3D spheroids post 6-bilayer encapsulation up to day 10 using alamar blue assay *(*p < 0.05, **p < 0.01 and ***p < 0.001),* Quantification of f) LDH release and g) Alamar blue intensities of the 6-bilayer nanoencapsulated 3D spheroids post 72 h incubation in cocktail stress media *(*p < 0.05, **p < 0.01 and ***p < 0.001),* and h) Bright-field and fluorescence microscopy images highlighting the shielding effect of the nanocoating in preventing the M1 macrophage infiltration up to 72 h (Scale bar: 100 μm).
2.8	Co-functionalization of tyramine (TA) along with EDA directly into the silk chain increased the stability of the nanocoating on the cell surface due to additional covalent interaction. a) Chemical synthesis of SF-EDA and SF-EDA-TA using carbodiimide coupling chemistry, b) Polyacrylamide gel image of SF-EDA and SF-EDA-TA with reference protein ladder, c) Quantification of mean and maximum molecular weight after co-functionalization of TA and EDA into the silk chains, d) Zeta potential measurements to estimate the change in overall charge of SF-EDA post-incorporation of TA into the silk chains *(*p < 0.05, **p < 0.01 and ***p < 0.001),* e, f and g) FTIR measurements to quantify the alteration in beta sheet content post co-functionalization of TA into the cationic silk ionomer *(*p < 0.05, **p < 0.01 and ***p < 0.001),* h) Water Contact Angle (WCA) measurements of SF-EDA and SF-EDA-TA *(*p < 0.05, **p < 0.01 and ***p < 0.001),* i) Fluorescence micrographs to compare the stability of the TA-functionalized nanocoatings on hMSCs with and without enzymatic crosslinking (Scale bar: 100 μm), j) Live/dead micrographs of the crosslinked and non-crosslinked TA-functionalized nanocoated hMSCs at days 1, 3 and 7, k) Comparing the cell proliferation rate of the hMSCs post-encapsulation with crosslinked and non-crosslinked TA-functionalized nanocoatings at days 1 and 7 respectively *(*p < 0.05, **p < 0.01 and ***p < 0.001),* and l,m)

	Confocal fluorescence micrographs and RBITC fluorescence intensity demonstrating the stability of the tyramine-functionalized nanocoating on the hMSCs in 3D spheroid platforms (Scale bar: 100 μm) *(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
Chapter 3	
3.1	Preliminary characterization of the synthesized SF(S)-GlcN and SF(D, E)-GlcN biopolymers, a) Graphical representation showing the chemical modification mechanism for incorporation of the glucosamine molecule into the SF side chains, b and c) SDS-PAGE gel image and the corresponding molecular weight quantification of the SF(S)-GlcN and SF(D, E)-GlcN modified as well as native (SF) and intermediate (SF-COOH) biopolymers, d) Live-dead micrographs of the day 28 hMSCs cultured on SF(S)-GlcN, SF(D, E)-GlcN, as well as native SF matrices (Scale bar: 100 μm), e) % cytocompatibility of the SF(S)-GlcN, SF(D, E)-GlcN and native SF matrices normalized to the 2D monolayer control (*(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
3.2	Assessing the structural stability of the SF-GlcN and G-TA composite hydrogel systems, a) Graphical representation showing the molecular mechanism behind the formation of stable hydrogel networks following enzymatic crosslinking with HRP/H_2O_2. b) Digital image of the inverted tube test depicting gelation in the SF(S)-GlcN-G-TA and SF(D,E)-GlcN-G-TA composite hydrogels, c and d) Digital image illustrating the stability of the SF-G-TA, SF(S)-GlcN-G-TA and SF(D,E)-GlcN-G-TA composite hydrogels following 28 days of chondrogenic differentiation, e and f) ATR-FTIR spectrum of the SF-G-TA, SF(S)-GlcN-G-TA and SF(D,E)-GlcN-G-TA composite hydrogels at days 1 and 28 and further deconvolution of the silk secondary conformation spectrum (1616 and 1637 cm^{-1}), g) Quantification of the % β-sheet, random coil, α-helix and β-turns of the composite hydrogels at days 1 and 28, and h) Bar graph representing the % β-sheet content of the respective composite hydrogels at days 1 and 28 as functional parameter for structural stability *(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
3.3	Rheological and morphological characterization of the SF-GlcN and G-TA composite hydrogels at different substitution ratios. Oscillatory strain sweep evaluation for a) SF-G/TA (1:1), b) SF/G-TA (1:3), c) SF(S)-GlcN/G-TA (1:1), d) SF(S)-GlcN/G-TA (1:3), e) SF(D, E)-GlcN/G-TA (1:1), f) SF(D, E)-GlcN/G-TA (1:3) composite hydrogels. Thixotropy characteristic analysis for g) SF/G-TA, h) SF(S)-GlcN/G-TA and i) SF(D, E)-GlcN/G-TA hydrogel systems at both substitution ratios. j) Representative injectability demonstration of SF(S)-GlcN/G-TA hydrogels at both 1:1 and 1:3 ratios and k) SEM micrographs for SF/G-TA, SF(S)-GlcN/G-TA and SF(D, E)-GlcN/G-TA hydrogel systems for both ratios (1:1 and 1:3) (Scale: 100 μm).
3.4	Rheological, morphological and immunological characterization of the SF-GlcN and G-TA composite hydrogels at different substitution ratios, Flow sweep analysis for a) SF-G-TA (1:1), b) SF-G-TA (1:3), c) SF(S)-GlcN-G-TA (1:1), d) SF(S)-GlcN-G-TA (1:3), e) SF(D, E)-GlcN-G-TA (1:1), f) SF(D, E)-GlcN-G-TA (1:3) composite hydrogels, g) Bar graph representation of mean

	pore diameter calculated from the SEM micrographs using Image J, h) Immune response profile of the SF-G-TA, SF(S)-GlcN-G-TA and SF(D, E)-GlcN-G-TA hydrogel systems using THP-1-ASC reporter cell line assay (Scale: 100 μm), and i) Quantification of the macrophage polarization ratio (M2:M1) from the fluorescence micrographs using Image J.
3.5	Investigating the cytocompatibility, immune response profile and the redox activity of the SF-GlcN and G-TA composite hydrogel systems across both ratios, a) Cell adhesion and morphology on the respective composite hydrogels, observed under a confocal microscope using actin cytoskeleton and nucleus staining. (Scale bar: 100 μm), b) Schematic representation showing the overall experimental design of *in vitro* biological characterization, c and d) Percentage cytocompatibility of the SF-GlcN and G-TA and non-glucosamine modified SF/G-TA composite hydrogel systems for both substitution ratios (1:1 and 1:3) at days 1 and 28, normalized to TCP control (monolayer) (*p < 0.05, **p < 0.01 and ***p < 0.001), e) Fluorescence micrograph showing the macrophage polarization profile of the respective SF-GlcN and G-TA and non-glucosamine modified SF/G-TA composite hydrogel systems for both substitution ratios (1:1 and 1:3). (Scale bar: 100 μm), f and g) Quantification of cleaved IL-1β from the THP-1 ASC-derived macrophages at 24 and 72 h (*p < 0.05, **p < 0.01 and ***p < 0.001) and h) Fluorescent micrographs depicting the GFP expression at days 1, 7 and 28 from the respective SF-GlcN and G-TA and non-glucosamine modified SF-G-TA composite hydrogels, as a functional parameter to evaluate the antioxidant activity of the hydrogel systems (Scale bar: 100 μm).
3.6	Quantifying the extent of ROS-scavenging (a-d), pro-chondrogenic (e,f) and anti-fibrotic (g) potential of the SF-GlcN and G-TA composite hydrogel systems for both substitution ratios (1:1 and 1:3) *(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
3.7	Assessing the *in vitro* chondrogenesis and fibrosis of the hMSCs cultured on SF-G-TA hydrogel system, Confocal micrographs demonstrating the expression profile of a, b) collagen type II (COL-II), c, d) aggrecan (ACAN), and e, f) collagen type I (COL-I) (Scale bar: 100 μm)
3.8	Assessing the *in vitro* chondrogenesis and fibrosis of the hMSCs cultured on SF(S)-GlcN-G-TA hydrogel system, Confocal micrographs demonstrating the expression profile of a, b) collagen type II (COL-II), c, d) aggrecan (ACAN), and e, f) collagen type I (COL-I) (Scale bar: 100 μm)
3.9	Assessing the *in vitro* chondrogenesis and fibrosis of the hMSCs cultured on SF(D, E)-GlcN-G-TA hydrogel system, Confocal micrographs demonstrating the expression profile of a, b) collagen type II (COL-II), c, d) aggrecan (ACAN), and e, f) collagen type I (COL-I) (Scale bar: 100 μm)
Chapter 4	
4.1	Computational modeling of TGFβ3 activation by conjugation with CyCl-modified SF heavy chain.

4.2	ATR-FTIR analysis a) Cyanuric Chloride modified different forms of silk fibroin (solution, film and hydrogel), b) Expanded 600-800 cm^{-1} region, and c) Expanded 1050-1250 cm^{-1} region.
4.3	Quantifying the secondary conformation of the SF biomaterial following chemical modification with cyanuric chloride (CyCl), a) Magnified 1590-1705 cm^{-1} region corresponding to the amide I region, b-e) Representative deconvoluted graphs of respective, untreated SF, SF-CyCl solution, SF-CyCl hydrogel and SF-CyCl film, and f) Bar graph demonstrating the quantification of secondary conformations of silk following CyCl modification
4.4	ATR-FTIR analysis of SF-CyCl-TGFβ, a) Full FTIR spectra of 2SF-CyCl-TGFβ (2% SF, 10%, 20% and 30% CyCl and 10 ng/ml TGFβ), b) Full FTIR spectra of 5SF-CyCl-TGFβ (5% SF, 10%, 20% and 30% CyCl and 10 ng/ml TGFβ) c-e) Expanded 600-800 cm^{-1} 2SF-CyCl-TGFβ and 5SF-CyCl-TGFβ regions, f-h) Expanded 2SF-CyCl-TGFβ and 5SF-CyCl-TGFβ 1050-1250 cm^{-1} regions, i-k) Expanded 2SF-CyCl-TGFβ and 2SF-CyCl-TGFβ 1550-1700 cm^{-1} regions
4.5	Quantifying the secondary conformation of the SF-CyCl solution following covalent conjugation with TGFβ3, a-c) Magnified 1595-1705 cm^{-1} region corresponding to the amide I region of 2% SF solution reacted with 10% 20% and 30% CyCl solution and 10 ng/ml of TGFβ3, d-f) Magnified 1595-1705 cm^{-1} region corresponding to the amide I region of 5% SF solution reacted with 10% 20% and 30% CyCl solution and 10 ng/ml of TGFβ3, and f) Bar graph demonstrating the quantification of secondary conformations of 2% and 5% SF following the chemically modification
4.6	^{1}H-NMR spectra, a) TGFβ3, b) CyCl modified SF, c) SF-CyCl-TGFβ trimeric conjugate.
4.7	CD spectra of TGFβ conjugated SF modified with CyCl with SF control.
4.8	a) SEM analysis of respective hBMSCs and hACs seeded on SF, SF with exogenous TGFβ addition and SF with TGFβ conjugated films, b) GAG estimation using DMMB assay for both hBMSCs and hACs; all experiments were carried out in triplicates (n=3), and the statistical analysis represented as * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$); the statistical analysis for both the experimental groups at day 28 were calculated against SF Day 28 control
4.9	Gene expression analysis for **hBMSCs**; all experiments were carried out in triplicates (n=3), and the statistical analysis represented as * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$); the statistical analysis for both the experimental groups at day 28 were calculated against SF Day 28 control

4.10	Gene expression analysis for **hACs;** all experiments were carried out in triplicates (n=3), and the statistical analysis represented as * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$); the statistical analysis for both the experimental groups at day 28 were calculated against SF Day 28 control
4.11	Histological analysis of the hBMSCs and hACs seeded on SF, SF with exogenous TGFβ addition and SF with TGFβ conjugated films at day 28. The statistical analysis of staining intensity quantification is represented as * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$) for both experimental groups compared to the SF control.
Chapter 5	
5.1	A-C) Computational modeling prediction of small molecule and growth factor conjugation with the chemically modified SF biomaterial, D) The model with the lowest C-score demonstrated stronger binding affinity, E-H) The Ramachandran plot illustrated the native conformation of the amino acid residues post-chemical modification of the SF biomaterial with Cyanuric Chloride (CyCl) and Diazonium salt (Diazo), respectively
5.2	ATR-FTIR characterization, A and B) Cyanuric chloride (CyCl) modification of SF biomaterial demonstrating peak at respective C-O-C bonding region, C-E) Diazonium coupling modification of SF biomaterial showing peaks at respective C-N bonding and S-H stretching regions, F-H) Deconvolution of the Amide-I region (1590-1710 cm^{-1}) for the respective SF-CyCl, SF-Diazo and SF (untreated) group, I) Quantification of the Beta-sheet content from the deconvoluted spectra.
5.3	A) UV visible spectroscopy of azo incorporation by diazonium coupling in SF biomaterial, B) Flow curve of the chemically modified SF-G bioinks showing characteristic shear-thinning behaviour of both the chemically decorated bioinks, C) Gelation kinetics of untreated mushroom tyrosinase crosslinked SF-G bioink, D) Gelation kinetics of mushroom tyrosinase crosslinked SF-G-CyCl bioink, E) Gelation kinetics of mushroom tyrosinase crosslinked SF-G-Diazo bioink, F-H) Optical microscopic images of the 3D constructs printed with respect acellular chemically modified SF-G bioinks, I) Quantification of the spreading ratio (n=5), J) Quantification of printability index (n=5), K) Quantification of the perimeter of the pore (n=5) and, L) Quantification of the area of the pore (n=5). Here, n = 5 corresponds to the evaluation of the respective parameter across 5 different regions.
5.4	A) Release profile of LDN193189 in chemically conjugated and encapsulated form, B) Release profile of TGFβ in chemically conjugated and encapsulated form, C) Release profile of IL1Ra in chemically conjugated and encapsulated form, D) Experimental timeline, E-G) 3D bioprinting and evaluation of bioprinted constructs at day 3 using Bright-field microscopy demonstrating perfect grid structure with homogenous cell distribution throughout the construct.

5.5	Evaluating the chondrogenesis profile of hBMSCs from three different donors primed with chondrogenic differentiation medium (ChM) for 14 days in monolayer against a proliferation medium (PfM) control A-C). Gene expression analysis of chondrogenic (COL2A1 and SOX9) and hypertrophic (COL10A1 and MMP-13) markers using qPCR for Donor-1, Donor-2 and Donor-3, respectively. The gene expression data were normalized to the values on day 1. D) Donor-to-donor variation of gene expression data, E) COL2A1 expression levels of the different donors, F-H) Alcian blue staining depicting enhanced GAG deposition in ChM samples than in PfM after 14 14-day culture period. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.6	**(**A-I): Gene expression analysis of chondrogenic (SOX9, PRG-4, and COL2A1), hypertrophic (COL10A1 and MMP-13), signaling pathway (SMAD-4, NOG, DKK1, and TGFβR1) markers of Donor-2 3D bioprinted constructs at day 28 of inflammatory culture incubation. The gene expression data were normalized to day 1 values; n.d.: not detectable. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.7	(A-H): Gene expression analysis of chondrogenic (SOX9, PRG-4, and COL2A1), hypertrophic (COL10A1 and MMP-13), signaling pathway (SMAD-4, NOG, and DKK1) markers of Donor-1 3D bioprinted constructs at day 28 of inflammatory culture incubation. The gene expression data were normalized to day 1 values; n.d.: not detectable. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.8	A-I): Gene expression analysis of chondrogenic (SOX9, PRG-4, and COL2A1), hypertrophic (COL10A1 and MMP-13), signaling pathway (SMAD-4, NOG, DKK1, and TGFβR1) markers of Donor-3 3D bioprinted constructs at day 28 of inflammatory culture incubation. The gene expression data were normalized to day 1 values; n.d.: not detectable. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.9	Biochemical estimation of A) proteoglycan content (DMMB), B) Total nitric oxide (TNO), C) Total nitrite content (Nitrite) and D) Lipid peroxidation profile from the Donor-2 3D bioprinted constructs and supernatants at day 28 of inflammatory culture incubation. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.10	Biochemical estimation of A) proteoglycan content (DMMB), B) Total nitric oxide (TNO), C) Total nitrite content (Nitrite) and D) Lipid peroxidation profile from the Donor-1 and Donor-3 3D bioprinted constructs and supernatants at day 28 of inflammatory culture incubation. All experiments were carried out in triplicate (n = 3), and the statistical analysis is represented

	as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.11	i(a-l) COL-II and ii(a-l) COL-X protein expression analysis of Donor-2 3D bioprinted constructs following incubation in OA-mimicking medium, using Immunofluorescence. The constructs were incubated with mouse anti-collagen II (5 µg/ml, DSHB) and mouse anti-collagen X (5 µg/ml, DSHB), followed by incubation with secondary antibody anti-mouse Alexa Fluor 488 (2 µg/ml, Thermo-fisher Scientific) and Hoechst (1 µg/ml, Thermo-fisher Scientific). The respective fluorescence intensity was calculated using ImageJ software. Measurements were taken from five different regions (n = 5), and the statistical analysis is represented as follows: * ($p \leq 0.033$), ** ($p \leq 0.002$), *** ($p \leq 0.001$), and **** ($p < 0.0001$).
5.12	Histological analysis of the Donor-2 3D bioprinted constructs following incubation in OA-mimicking medium for 28 days. i(a-h) Safranin O staining, ii(a-h): Alcian blue staining and iii(a-d): Hematoxylin and eosin staining (H&E).
5.13	Gene Mania analysis depicting the correlation and interaction of the input genes with the pool of genes associated with articular cartilage homeostasis. Five upregulated genes (from qPCR analysis) of each experimental group of Donor-2 were selected for the interaction study. (**SF-G-IL1Ra**: *NOG, PRG4, TGFβR1, COL2A1 & SOX9*; **SF-G-TGFβ:** *COL10A1, DKK1, MMP13, SMAD4 & COL2A1*; **SF-G-LDN**: *NOG, COL10A1, COL2A1, SOX9 & SMAD4*; **SF-G:** *PRG4, COL10A1, DKK1, MMP13 & SMAD4*)
Chapter 6	
6.1	Physicochemical characterization of the chemically modified SF-G constructs a) Schematic representation of *in vitro* physical, mechanical and biological characterization of 3D bioprinted LDN-193189 SF-G constructs, b) Representative images of 3D bioprinted chemically modified SF-G constructs with rat BMSCs, c) Representative microscopic image of 3D bioprinted SF-G-LDN constructs before (day 1) and after (day 14) degradation, d) Comparative degradation profile of SF-G constructs with and without LDN conjugation, e) Representative microscopic image of 3D bioprinted SF-G-LDN constructs before (day 1) and after (day 14) swelling, f) Comparative swelling profile of SF-G constructs with and without LDN conjugation, g,h, i) Compressibility and recoverability of LDN-conjugated 3D bioprinted SF-G constructs and j) AFM analysis of chemically modified and unmodified SF-G films.
6.2	*In vitro* biological characterization of the 3D bioprinted SF-G-LDN-193189 constructs. a) Immunofluorescence analysis of collagen II expression in LDN-conjugated and control 3D bioprinted SF-G constructs, b) Immunofluorescence analysis of collagen X expression in LDN-conjugated and control 3D bioprinted SF-G constructs, c) Safranin O staining in LDN-conjugated and control 3D bioprinted SF-G constructs, d) Gene expression analysis of chondrogenic and hypertrophic marker genes in LDN-conjugated

	and control 3D bioprinted SF-G constructs, and e) Biochemical estimation in LDN-conjugated and control 3D bioprinted SF-G constructs *(*p < 0.05, **p < 0.01 and ***p < 0.001)*.
6.3	LDN-193189-conjugated SF-G constructs promote site-specific cartilage repair and proteoglycan restoration in cartilage defects in the rat OA knee joint. a) Schematic representation of the experimental strategy for implantation of SF-G-LDN-193189 conjugated constructs in a full-thickness cartilage defect model post-ACLT day 28. b) (i-viii): Gross morphology of the knee joint depicting various experimental groups post 2 months and 4 months implantation. c) (i-viii): 3D rendering volumetric micro-CT scanning of knee joints depicting the experimental groups. d) (i-iv): Histopathological (Safranin O/Fast Green) staining of post-implantation 4 months; e) Blind OARSI scoring, Brown-Forsythe test (ANOVA), and Tukey's multiple comparison tests were performed to assess the significance and simultaneous comparison of the multiple sets. We compared the means of Unmanipulated vs. ACLT+ Defect+ No constructs group, p=0.0001(***); ACLT+ Defect + No constructs group vs. ACLT + Defect+ SF-G-LDN-193189, p=0.0009(***); and ACLT+Defect +SF-G vs. ACLT + Defect+ SF-G-LDN-193189, p=0. 0009(***). Scale bar: 200 μm.
6.4	LDN-193189-conjugated 3D printed constructs inhibited BMP signaling pathway in cartilage defects in the rat OA knee joint. a) Dual Luciferase reporter assay (BRITER), b (i-iv): pSMAD1/5/9 immunostaining of rat knee joint post 4 months implantation for different experimental groups; Scale bar: 50μm.
6.5	LDN-193189-conjugated 3D printed constructs promote regeneration of hyaline cartilage and suppress hypertrophy in cartilage defects in the rat OA knee joint. a) (i-iv) ColII immunostaining of rat knee joint of post 2-months implantation and a) (v-viii) post 4-months implantation for different experimental groups; b) (i-iv) ColX immunostaining of rat knee joint of post 2-months implantation and b) (v-viii) post 4-months implantation for different experimental groups; c) (i-iv) ColI immunostaining of rat knee joint of post 2-months implantation for different experimental groups and c) (v-viii) post 4-months implantation. White boxes indicate the site of the created defect area; scale bar: 50 μm.
6.6	LDN-193189 Conjugated 3D printed constructs attenuate inflammatory pathways to create a pro-regenerative niche in cartilage defects in the rat OA knee joint. a) (i-iv') NFκB immunostaining of the rat knee joint of post 2-months implantation and a) (v-viii') post 4-months of implantation for different experimental groups. White dashed boxes mark the created defect site; Scale bar: 50μm

List of Tables

1.1	Hypotheses regarding the onset of chondrocyte hypertrophy
3.1	β-sheet content of the composite hydrogels at days 1 and 28
3.2	Viscosity drop from the Flow Sweep analysis
3.3	Storage modulus recovery following shear deformation
4.1	Model descriptions for SF-CyCl-TGFβ molecular modeling
4.2	Experimental sample description
4.3	List of analyzed genes using RT PCR
5.1	List of analyzed genes using RT PCR
5.2	Quantification of % tyrosine residues modification of SF upon diazonium coupling
6.1	Experimental group
6.2	Antibody biomarkers and their role in cartilage homeostasis