

ENGINEERING MULTIFUNCTIONAL HYDROGELS FOR ENHANCED CARTILAGE REGENERATION

Shivani Chaudhary



Centre for Biomedical Engineering

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by

Shivani Chaudhary

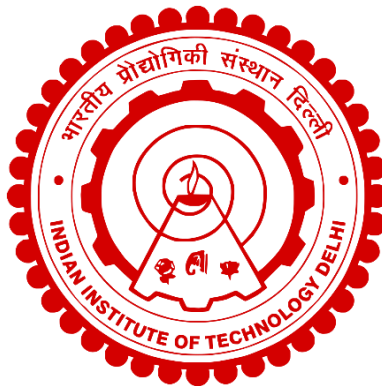
Centre for Biomedical Engineering

Submitted

In fulfillment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

July 2026

In loving memory of my mother

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This is to certify that the thesis entitled “**Engineering multifunctional hydrogels system for enhanced cartilage regeneration**” has been carried out by **Ms. Shivani Chaudhary**, a bonafide **Ph.D.** student at Centre for Biomedical Engineering, Indian Institute of Technology Delhi. The conceptualization, investigations, observations, and conclusions reported in the thesis are based on her original work conducted under my direct supervision. This thesis, being submitted for the award of Ph.D. degree has not been submitted in part or in full to this or any other University for the award of any degree or diploma.

Date: July 2026

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Abstract

Articular cartilage is a specialized connective tissue that provides a smooth and lubricated surface for joint movement while distributing mechanical loads during locomotion. However, due to its avascular, aneural, and alymphatic nature, cartilage possesses extremely limited intrinsic healing capacity. Once damaged by trauma, aging, or disease, the tissue undergoes progressive degeneration characterized by extracellular matrix breakdown, loss of chondrocyte function, inflammation, and deterioration of mechanical integrity. If left untreated, such damage can eventually lead to a debilitating joint disorder known as Osteoarthritis (OA). Current clinical interventions including microfracture, autologous chondrocyte implantation, and osteochondral grafting often provide only temporary relief and rarely succeed in restoring the complex structure and functionality of native cartilage.

In recent years, biomaterial-based strategies have emerged as promising approaches for cartilage repair. However, many existing scaffold systems fail to achieve long-term regeneration because they do not adequately replicate three essential characteristics of native cartilage: biomimetic structural architecture, sufficient mechanical strength to withstand joint loading, and metabolic support necessary for chondrocyte survival and differentiation. Furthermore, the joint microenvironment in degenerative conditions is often hypoxic, inflammatory, and metabolically compromised, which further limits cell viability and extracellular matrix synthesis. These challenges highlight the need for multifunctional biomaterials capable of actively supporting cellular metabolism, promoting tissue integration, and sustaining long-term cartilage regeneration. The central objective of this thesis is to address the major biological and mechanical barriers associated with cartilage regeneration by designing advanced hydrogel-based biomaterials that integrate biomimetic architecture, mechanical resilience, and bioenergetic functionality. To achieve this goal, a series of innovative scaffold systems were developed, each targeting a specific challenge in cartilage tissue engineering.

Chapter 1 provides a comprehensive introduction and literature survey discussing cartilage structure, degeneration mechanisms, and current regenerative strategies for treating osteoarthritis.

Chapter 2 describes the development of a Janus nanofiber-reinforced biomimetic fibrillar hydrogel, designed to replicate the hierarchical dual architecture of native cartilage. This system integrates microporous and nanofibrous features mimicking the structural complexity of natural cartilage tissue to enhance strength of scaffold and promote cell bioactivity, and chondrogenic differentiation.

Chapter 3 presents **BioEnerGel**, a robust, self-healable, and tissue-adhesive hydrogel engineered to provide improved mechanical strength, structural stability, and effective tissue integration under load-bearing conditions. This multifunctional hydrogel platform incorporates bioenergetic components to address the metabolically restrictive microenvironment of cartilage by enhancing cellular metabolism and supporting chondrogenesis under hypoxic conditions.

Chapter 4 presents **OxyCartiGel**, an oxygen-releasing bioenergetic hydrogel platform developed to address the severe hypoxic microenvironment associated with cartilage injury and degeneration. By providing sustained oxygen release, this system aims to meet the oxygen dependent metabolic demands of chondrocytes and stem cells, thereby improving cell survival, promoting extracellular matrix synthesis, and supporting effective cartilage regeneration.

Finally, **Chapter 5** summarizes the key findings of this work and outlines future perspectives for the development of next-generation multifunctional biomaterials for cartilage regeneration. Collectively, this thesis advances the design of bioinspired hydrogel systems that synergistically integrate structural, mechanical, and metabolic cues, offering a promising strategy for effective cartilage repair and the prevention of osteoarthritis progression.

सार

आर्टिकुलर कार्टिलेज एक विशेष प्रकार का संयोजी ऊतक है जो जोड़ों की गति के लिए एक चिकनी तथा स्नेहयुक्त सतह प्रदान करता है और चलने-फिरने के दौरान उत्पन्न होने वाले यांत्रिक भार का वितरण करता है। हालांकि, अपनी अवस्कुलर (रक्तवाहिकाओं का अभाव), एनीयूरल (तंत्रिकाओं का अभाव) तथा एलिम्फैटिक (लसीका तंत्र का अभाव) प्रकृति के कारण कार्टिलेज की स्वाभाविक उपचार क्षमता अत्यंत सीमित होती है। जब यह ऊतक चोट, बढ़ती आयु अथवा किसी रोग के कारण क्षतिग्रस्त हो जाता है, तो इसमें क्रमिक अपक्षय प्रारम्भ हो जाता है। इस अपक्षय की पहचान बाह्यकोशिकीय मैट्रिक्स के विघटन, कोंड्रोसाइट कार्यक्षमता में कमी, सूजन तथा यांत्रिक अखंडता में गिरावट से होती है। यदि समय पर इसका उपचार न किया जाए, तो यह स्थिति अंततः ऑस्टियोआर्थराइटिस (Osteoarthritis, OA) नामक जोड़ों की एक गंभीर और दुर्बलकारी बीमारी में परिवर्तित हो सकती है। वर्तमान नैदानिक उपचार विधियाँ जैसे माइक्रोफ्रैक्चर, ऑटोलॉग्स कोंड्रोसाइट प्रत्यारोपण तथा ऑस्टियोकॉन्ड्रल ग्राफ्टिंग अक्सर केवल अस्थायी राहत प्रदान करती हैं और प्राकृतिक कार्टिलेज की जटिल संरचना एवं कार्यक्षमता को पूर्ण रूप से पुनर्स्थापित करने में प्रायः सफल नहीं हो पातीं। हाल के वर्षों में, बायोमैटेरियल आधारित रणनीतियाँ कार्टिलेज मरम्मत के लिए आशाजनक दृष्टिकोण के रूप में उभरी हैं। तथापि, कई मौजूदा स्कैफोल्ड प्रणालियाँ दीर्घकालिक पुनर्जनन प्राप्त करने में असफल रहती हैं क्योंकि वे प्राकृतिक कार्टिलेज की तीन महत्वपूर्ण विशेषताओं को पर्याप्त रूप से पुनरुत्पादित नहीं कर पातीं: जैव-अनुकरणीय संरचनात्मक वास्तुकला, जोड़ों पर पड़ने वाले भार को सहन करने हेतु पर्याप्त यांत्रिक मजबूती, तथा कोंड्रोसाइट के जीवित रहने और विभेदन के लिए आवश्यक चयापचय समर्थन। इसके अतिरिक्त, अपक्षयी परिस्थितियों में जोड़ों का सूक्ष्म पर्यावरण प्रायः हाइपोक्सिक, सूजनयुक्त तथा चयापचय की दृष्टि से बाधित होता है, जिससे कोशिकीय जीवितता तथा बाह्यकोशिकीय मैट्रिक्स संश्लेषण और अधिक सीमित हो जाता है। ये चुनौतियाँ ऐसे बहु-कार्यात्मक बायोमैटेरियल्स की आवश्यकता को रेखांकित करती हैं जो सक्रिय रूप से कोशिकीय चयापचय का समर्थन कर सकें, ऊतक एकीकरण को बढ़ावा दें तथा दीर्घकालिक कार्टिलेज पुनर्जनन को बनाए रख सकें। इस थीसिस का मुख्य उद्देश्य उन्नत हाइड्रोजेल आधारित बायोमैटेरियल्स का विकास करके कार्टिलेज पुनर्जनन से संबंधित प्रमुख जैविक तथा यांत्रिक बाधाओं को दूर करना है। इन बायोमैटेरियल्स को इस प्रकार डिजाइन किया गया है कि वे जैव-अनुकरणीय संरचना, यांत्रिक दृढ़ता तथा जैव-ऊर्जात्मक कार्यक्षमता को एकीकृत कर सकें। इस लक्ष्य की प्राप्ति हेतु विभिन्न नवोन्मेषी स्कैफोल्ड प्रणालियाँ विकसित की गईं, जिनमें से प्रत्येक कार्टिलेज ऊतक अभियांत्रिकी की एक विशिष्ट चुनौती को संबोधित करती है।

अध्याय 1 में कार्टिलेज की संरचना, उसके अपक्षय के तंत्र तथा ऑस्टियोआर्थराइटिस के उपचार के लिए वर्तमान पुनर्जनन रणनीतियों पर आधारित विस्तृत परिचय एवं साहित्य समीक्षा प्रस्तुत की गई है।

अध्याय 2 में जेनस नैनोफाइबर-सुदृढ़ित जैव-अनुकरणीय फाइब्रिलर हाइड्रोजेल के विकास का वर्णन किया गया है, जिसे प्राकृतिक कार्टिलेज की पदानुक्रमिक द्वि-संरचना की नकल करने के लिए डिजाइन किया गया है। यह प्रणाली माइक्रोपोरस तथा नैनोफाइबर विशेषताओं को एकीकृत करती है, जो प्राकृतिक कार्टिलेज ऊतक की संरचनात्मक जटिलता का अनुकरण करती हैं। इससे स्कैफोल्ड की यांत्रिक मजबूती बढ़ती है तथा कोशिकीय जैव-सक्रियता और कोंड्रोजेनिक विभेदन को प्रोत्साहन मिलता है।

अध्याय 3 में **बायोएनर्जेल** प्रस्तुत किया गया है, जो एक सुदृढ़, स्व-उपचारणीय तथा ऊतक-आसंजन हाइड्रोजेल है। इसे भार-वहन परिस्थितियों में बेहतर यांत्रिक मजबूती, संरचनात्मक स्थिरता तथा प्रभावी ऊतक एकीकरण प्रदान करने के लिए अभिकल्पित किया गया है। यह बहु-कार्यात्मक हाइड्रोजेल प्लेटफॉर्म जैव-ऊर्जात्मक घटकों को सम्मिलित करता है, जो कार्टिलेज के चयापचय-सीमित सूक्ष्म पर्यावरण को संबोधित करते हुए कोशिकीय चयापचय को बढ़ाते हैं तथा हाइपोक्सिक परिस्थितियों में कोंड्रोजेनेसिस का समर्थन करते हैं।

अध्याय 4 में **ऑक्सीकार्टिजेल** नामक एक ऑक्सीजन-रिलीजिंग जैव-ऊर्जात्मक हाइड्रोजेल प्लेटफॉर्म प्रस्तुत किया गया है, जिसे कार्टिलेज की चोट और अपक्षय से जुड़े गंभीर हाइपोक्सिक सूक्ष्म पर्यावरण को संबोधित करने के लिए विकसित किया गया है। सतत ऑक्सीजन विमोचन प्रदान करके यह प्रणाली कोंड्रोसाइट्स तथा स्टेम कोशिकाओं की ऑक्सीजन-निर्भर चयापचय आवश्यकताओं को पूरा करने का प्रयास करती है। इससे कोशिका जीवितता में सुधार, बाह्यकोशिकीय मैट्रिक्स संश्लेषण में वृद्धि तथा प्रभावी कार्टिलेज पुनर्जनन को समर्थन मिलता है।

अंततः, **अध्याय 5** इस शोध कार्य के प्रमुख निष्कर्षों का सार प्रस्तुत करता है तथा कार्टिलेज पुनर्जनन के लिए अगली पीढ़ी के बहु-कार्यात्मक बायोमैटेरियल्स के विकास की भविष्य संभावनाओं पर प्रकाश डालता है। समग्र रूप से, यह थीसिस जैव-प्रेरित हाइड्रोजेल प्रणालियों के डिजाइन को आगे बढ़ाती है, जो संरचनात्मक, यांत्रिक तथा चयापचय संकेतों को समन्वित रूप से एकीकृत करती हैं और प्रभावी कार्टिलेज मरम्मत तथा ऑस्टियोआर्थराइटिस की प्रगति को रोकने के लिए एक आशाजनक रणनीति प्रस्तुत करती हैं।

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Abbreviations

ECM: Extracellular matrix

ATP: Adenosine triphosphate

OA: Osteoarthritis

SZ: Superficial zone

MZ: Middle zone

DZ: Deep zone

CCZ: Calcified Cartilage Zone

MMP: Matrix metalloproteinases

DAMPs: Damage-associated molecular patterns

BMI: Body mass index

K&L: Kellgren and Lawrence

COX-2: Cyclooxygenase-2

NSAIDs: Non-steroidal anti-inflammatory drugs

MN: Microneedle

COL-II: Type II collagen

ALD: Arthroscopic lavage and debridement

OAT: Osteochondral autograft transplantation

MACI: Matrix-Assisted Autologous Chondrocyte Implantation

HA: Hyaluronic acid

GAG: Glycosaminoglycan

ROS: Reactive oxygen species

Micro-CT: Micro-computed tomography

PG: Proteoglycan

AMPK: AMP-activated protein kinase

MgO: Magnesium oxide

TCA: Tricarboxylic acid

HSP70: Heat shock protein 70

Gln: Glutamine

ZC: Zonal chondrocytes

MSCs: Mesenchymal stem cells

DLP: Digital light processing (DLP)

MiMP: Mitochondrial membrane potential