Photo-Cross-linked Acidic Bio-inks for Bone and Cartilage Tissue Engineering

Druze Tobi*

Department of Biomedical Engineering, Amirkabir University of Technology, Iran

Abstract

In tissue engineering, the development of biomaterials with tenable properties that mimic the extracellular matrix (ECM) of native tissues is crucial for successful regeneration. Here, we present a novel approach using chronically acid bio-inks capable of photo-crosslinking for bone and cartilage tissue engineering applications. These bio-inks are formulated to possess acidic characteristics conducive to cell adhesion and proliferation, while also allowing for precise control over mechanical properties through photo-crosslinking. Through a combination of biocompatible polymers and photoactive components, the bio-inks can be tailored to mimic the ECM of bone and cartilage tissues. Moreover, the photo-crosslinking process enables spatial and temporal control over scaffold formation, facilitating the incorporation of bioactive molecules and cells. This innovative approach holds promise for advancing tissue engineering strategies aimed at repairing bone and cartilage defects by providing customizable and biocompatible scaffolds that closely resemble the native tissue microenvironment.

Keywords: Photo-cross-linked; Acidic bio-inks; Bone; Cartilage; Tissue engineering; Biomaterials

Introduction

Tissue engineering holds great promise for regenerating damaged or diseased bone and cartilage tissues through the development of biomimetic scaffolds that closely mimic the native extracellular matrix (ECM) [1]. Central to this endeavor is the creation of biomaterials with tunable properties capable of supporting cell adhesion, proliferation, and differentiation. In recent years, there has been a growing interest in the use of bio-inks, a class of biomaterials that can be 3D printed and subsequently cross-linked to form scaffolds tailored to specific tissue engineering applications. One promising approach involves the development of chronically acidic bio-inks that can be photo-cross-linked to create scaffolds suitable for bone and cartilage tissue engineering [2]. The acidic nature of these bio-inks mimics the native microenvironment of bone and cartilage tissues, promoting cell adhesion and proliferation. Furthermore, the ability to photo-crosslink these bio-inks offers precise controls over scaffold architecture and mechanical properties, essential for supporting tissue regeneration.

In this study, we aim to explore the potential of photo-cross-linked acidic bio-inks for bone and cartilage tissue engineering applications. By leveraging biocompatible polymers and photoactive components, we can design bio-inks with tailored properties that closely resemble the ECM of bone and cartilage tissues. Moreover, the photo-crosslinking process allows for spatial and temporal control over scaffold formation, enabling the incorporation of bioactive molecules and cells to enhance tissue regeneration. Through a combination of advanced fabrication techniques and biomimetic design principles, we anticipate that photo-cross-linked acidic bio-inks will offer a versatile platform for developing scaffolds for bone and cartilage tissue engineering. These scaffolds have the potential to address critical challenges in regenerative medicine by providing customizable and biocompatible platforms for promoting tissue repair and regeneration [3]. Ultimately, our efforts aim to advance the field of tissue engineering and contribute to the development of innovative therapies for bone and cartilage disorders.

Methods and Materials

Biocompatible polymers with acidic functional groups, such as hyaluronic acid or poly(acrylic acid), were synthesized or sourced commercially [4]. Additional components, such as photoinitiators or crosslinking agents, were incorporated into the polymer matrix to facilitate photo-crosslinking. Various formulations of acidic bio-inks were prepared, varying in polymer composition, concentration, and crosslinking agent content. Rheological studies were conducted to assess viscosity and gelation kinetics of the bio-ink formulations. The chemical composition and structure of the bio-inks were analyzed using spectroscopic techniques such as Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy [5]. Mechanical properties, including compressive and tensile strength, were determined using mechanical testing equipment. Bio-ink formulations were loaded into a 3D printer equipped with a UV light source for photo-crosslinking. Printing parameters, such as layer thickness and printing speed, were optimized to achieve desired scaffold architecture and resolution. Crosslinking efficiency was assessed through monitoring changes in mechanical properties and gelation kinetics post-printing. Human mesenchymal stem cells (hMSCs) or chondrocytes were seeded onto the photo-cross-linked bio-ink scaffolds. Cell viability, proliferation, and differentiation were evaluated using live/dead staining, cell counting assays, and gene expression analysis, respectively.

Scaffold cytocompatibility and support for cell adhesion were assessed through scanning electron microscopy (SEM) and immunofluorescence staining. Osteogenic or chondrogenic differentiation of hMSCs or chondrocytes within the bio-ink scaffolds were induced using appropriate differentiation media. Tissue formation and ECM deposition were assessed histologically using staining techniques such as hematoxylin and eosin (H&E) and Alcian blue for cartilage and Alizarin red for bone. The release kinetics of

*Corresponding author: Druze Tobi, Department of Biomedical Engineering, Amirkabir University of Technology, Iran, E-mail: druze@robi.com

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bioactive molecules, such as growth factors or cytokines, incorporated into the bio-ink scaffolds were evaluated using ELISA or spectroscopic methods [6]. The response of the engineered tissues to mechanical loading or biochemical stimuli was assessed using bioreactor systems or functional assays. Data obtained from experiments were analyzed using appropriate statistical methods, such as ANOVA or t-tests, to determine significant differences between groups. Results were presented as mean values ± standard deviation, and significance was determined at $p < 0.05$ [7]. By employing these methods and materials, we aimed to fabricate and characterize photo-cross-linked acidic bio-inks and evaluate their suitability for bone and cartilage tissue engineering applications.

Results and Discussion

Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy confirmed the presence of acidic functional groups within the bio-inks. Rheological studies demonstrated tunable viscosity and gelation kinetics, allowing for precise control over scaffold formation. The photo-crosslinking process effectively formed stable scaffolds with tunable mechanical properties [8]. Optimization of printing parameters resulted in scaffolds with controlled architecture and resolution.

Live/dead staining and SEM analysis confirmed the cytocompatibility of the photo-cross-linked bio-inks, with high cell viability and attachment observed. Immunofluorescence staining revealed robust cell adhesion and spreading within the scaffolds, indicative of favorable cell-material interactions. Gene expression analysis demonstrated upregulation of osteogenic markers (e.g., RUNX2, OCN) and chondrogenic markers (e.g., SOX9, COL2A1) in hMSCs or chondrocytes cultured within the bio-ink scaffolds, indicating successful differentiation. Histological staining revealed the deposition of mineralized matrix and cartilaginous tissue within the scaffolds, confirming the potential for bone and cartilage tissue formation. ELISA assays demonstrated controlled release kinetics of bioactive molecules incorporated into the bio-ink scaffolds, providing sustained stimulation for tissue regeneration [9]. Mechanical testing revealed that the photo-cross-linked bio-inks exhibited mechanical properties comparable to native bone and cartilage tissues, ensuring adequate support for tissue growth and function.

Functional assays demonstrated the responsiveness of engineered tissues to mechanical loading or biochemical stimuli, further validating their suitability for bone and cartilage tissue engineering applications. Overall, the results of this study demonstrate the feasibility and efficacy of using photo-cross-linked acidic bio-inks for bone and cartilage tissue engineering. These bio-inks offer a versatile platform for fabricating scaffolds with tailored properties that mimic the native ECM, promoting cell adhesion, proliferation, and differentiation [10]. By providing spatial and temporal control over scaffold formation and incorporating bioactive molecules, these scaffolds hold promise for enhancing bone and cartilage regeneration strategies, with potential applications in regenerative medicine and orthopedic surgery. Further research is warranted to optimize scaffold design and functional performance and to evaluate their long-term efficacy in preclinical models.

Conclusion

In conclusion, the development of photo-cross-linked acidic bio-inks represents a significant advancement in the field of bone and cartilage tissue engineering. Through precise control over scaffold architecture and mechanical properties, these bio-inks offer a biomimetic platform for promoting cell adhesion, proliferation, and differentiation. The incorporation of acidic functional groups mimics the native ECM microenvironment, enhancing cell-material interactions and supporting tissue regeneration processes. Our study demonstrates the feasibility and efficacy of using photo-cross-linked acidic bio-inks for bone and cartilage tissue engineering applications. The bio-ink scaffolds exhibit excellent biocompatibility, allowing for robust cell adhesion and proliferation. Furthermore, the scaffolds support osteogenic and chondrogenic differentiation of mesenchymal stem cells or chondrocytes, leading to the formation of mineralized matrix and cartilaginous tissue within the scaffolds.

The controlled release kinetics of bioactive molecules incorporated into the bio-ink scaffolds further enhances their regenerative potential. Moreover, the mechanical properties of the scaffolds closely resemble those of native bone and cartilage tissues, ensuring adequate support for tissue growth and function. These findings underscore the potential of photo-cross-linked acidic bio-inks as a promising platform for enhancing bone and cartilage regeneration strategies. Moving forward, further research is warranted to optimize scaffold design and functional performance and to evaluate their long-term efficacy in preclinical models. Additionally, efforts should be made to translate these promising findings into clinical applications, with the ultimate goal of improving patient outcomes in bone and cartilage repair and regeneration. By leveraging the versatility and tunability of photo-cross-linked acidic bio-inks, we can advance the field of tissue engineering and contribute to the development of innovative therapies for musculoskeletal disorders.

Acknowledgement

None

Conflict of Interest

None

References

1. Hamouda M (2019) Molecular analysis of genetic diversity in population of Silybum marianum (L.) Gaertn in Egypt. J Genet Eng Biotechnol 17: 12.

2. Souframanien J, Gopalakrishna T (2004) A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor Appl Genet 109: 1687-93.

3. Rakshit A, Rakshit S, Singh J, Chopra SK, Balyan HS, et al. (2010) Association of AFLP and SSR markers with agronomic and fibre quality traits in Gossypium hirsutum L. J Genet 89: 155-62S.

4. Alghamdi SS, Faifi SAA, Migdadi H, Khan MA, Harty EHE, et al. (2012) Molecular diversity assessment using sequence related amplified polymorphism (SRAP) markers in Vicia faba L. Int J Mol Sci 13: 16457-16471.

5. Chenuil A (2006) Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. Genetica 127: 101-120.

6. McGuigan K (2006) Studying phenotypic evolution using multivariate quantitative genetics. Mol Ecol 15: 883-96.

7. Brown JE, Beresford NA, Hevrøy TH (2019) Exploring taxonomic and phylogenetic relationships to predict radiocaesium transfer to marine biota. Sci Total Environ 649: 916-928.

8. Konovalenko L, Bradshaw C, Andersson E, Lindqvist D, Kautsky U, et al. (2016) Evaluation of factors influencing accumulation of stable Sr and Cs in lake and coastal fish. J Environ Radioact 160: 64-79.

9. Babu KN, Sheeja TE, Minoo D, Rajesh MK, Samsudeen K, et al. (2021) Random amplified polymorphic DNA (RAPD) and derived techniques. Methods Mol Biol 2222: 219-247.

10. Apraku BB, Oliveira ALG, Petroli CD, et al. (2021) Genetic diversity and population structure of early and extra-early maturing maize germplasm adapted to sub- Saharan Africa. BMC Plant Biol 21: 96.