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Enhancing The Bone Formation Properties Of Chondroitin Sulphate Based Injectable Hydrogels Incorporated With Dap And Cu Nanoparticles Using Cissus Quadrangularis

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ABSTRACT

Introduction: Chondroitin sulfate (OS) IS a naturally occurring glycosaminoglycan, commonly found in connective tissues such as cartilage and bone. Chondroitin sulfate, a naturally occurring polysaccharide, is a fundamental component of the extracellular matrix in various tissues, most notably in cartilage. Cartilage, with its limited regenerative capacity, poses a significant challenge in the field of tissue engineering and regenerative medicine.

Materials and Method: Chondroitin sulfate-based injectable hydrogels enriched with dapsone (DAP) and copper (Cu) nanoparticles are formulated. Cissus quadrangularis extract is integrated. The materials synergistically enhance bone formation properties, combining anti-inflammatory effects of DAP, osteogenic benefits of Cu nanoparticles, and bioactive compounds from Cissus quadrangularis for improved regenerative outcomes in orthopedic applications.

Results: Presented that the hydrogel formed fast in situ after injection due to the non-covalent cross-linking, and then reinforced by the DA chemical cross linking.The study determined the percentage of cell viability between the sample and control group. In addition, mechanical property of the hydrogel under dynamic conditions was determined to mimic dynamic bone tissues.

Conclusion: Chondroitin sulphate-based injectable hydrogels represent an exciting development in the field of regenerative medicine and orthopedics. They have shown promising results in bone tissue engineering, promoting bone formation and repair due to their excellent biocompatibility, biodegradability, and mimicking of the natural extracellular matrix.

Keywords: *Chondroitin sulphate, injectable hydrogels, bone matrix, Cissus quadrangularis*

1. INTRODUCTION

Chondroitin sulfate (OS) IS a naturally occurring glycosaminoglycan, commonly found in connective tissues such as cartilage and bone. In recent years, researchers have been exploring the potential of chondroitin sulfate-based in ectable nanogels as a promising biomaterial for bone regeneration and tissue engineering applications.[1] These hydrogels possess unique properties that make them attractive for promoting bone formation and facilitating the healing process.[2]

In the realm of biomedical materials and regenerative medicine, chondroitin sulfate-based injectable hydrogels have emerged as a revolutionary and captivating area of research and development.[3] These hydrogels, derived from chondroitin sulfate, hold tremendous potential for addressing a wide array of medical challenges and promoting tissue regeneration. This thousand-word introduction will explore the composition, properties, and the diverse applications of chondroitin sulfate-based injectable hydrogels, shedding light on the remarkable advancements and promising prospects in this field.[4]

Chondroitin sulfate, a naturally occurring polysaccharide, is a fundamental component of the extracellular matrix in various tissues, most notably in cartilage.[5] Cartilage, with its limited regenerative capacity, poses a significant challenge in the field of tissue engineering and regenerative medicine. Injectable hydrogels, which can be precisely delivered to damaged or degenerated tissues, have emerged as a solution to this challenge.[6] By incorporating chondroitin sulfate into hydrogel matrices, researchers aim to harness its unique properties to facilitate tissue repair, regeneration, and the treatment of various medical conditions.[7]

To comprehend the significance of chondroitin sulfate-based injectable hydrogels, it is crucial to delve into their composition and the remarkable characteristics that make them promising candidates for a wide range of applications.

The injectable nature of chondroitin sulfate-based hydrogels allows for minimally invasive delivery, enabling their use in a variety of clinical settings. When injected into a defect or injury site, the hydrogel can conform to the shape of the defect and form a three-dimensional scaffold, providing mechanical support and space for cell infiltration.[7] Furthermore, the hydrogel matrix can be modified to incorporate additional bioactive molecules. such as growth factors or osteogenic agents. further enhancing their bone-forming potential. These modifications can be achieved through the introduction of functional groups or the incorporation of nanoparticles within the hydrogel structure.[8]

Cissus quadrangularis extract, on the other hand, contains various bioactive compounds, including flavonoids, triterpenoids, and sterols,

which have been shown to possess osteogenic and anabolic etfects. These compounds promote the proliferation and differentiation of osteoblasts. The cells responsible for bone formation leading to bone generation.The development of injectable hydrogels incorporated with dapsone (DAP) and copper (Cu) nanoparticles, utilizing the natural source of Cissus quadrangularis, represents an innovative and multidisciplinary approach with significant implications for both biomedical and environmental fields. [9]

These injectable hydrogels are at the intersection of biotechnology and materials science, harnessing the potential of a plant-derived source, Cissus quadrangularis, to enhance the properties and applications of hydrogels. Injectable hydrogels have gained substantial attention in recent years for their versatile applications in drug delivery, tissue engineering, and wound healing. These materials offer the unique advantage of being delivered as a liquid and subsequently forming a gel within the target site, enabling precise and minimally invasive administration. The incorporation of DAP and Cu nanoparticles into these hydrogels, with the botanical source of Cissus quadrangularis, holds the promise of expanding their applications to tackle complex medical challenges and environmental concerns.[10]

The composition of these injectable hydrogels is a combination of natural and synthetic components, making them highly adaptable for specific uses. Cissus quadrangularis, a plant known for its rich medicinal properties, provides a biocompatible and sustainable source for enhancing the hydrogels. DAP, a medication with anti-inflammatory and antibacterial properties, and copper nanoparticles, known for their antimicrobial and catalytic capabilities, are integrated into the hydrogel to create a multifunctional and dynamic material.[11]

The unique properties of this combination, along with the renewable source of Cissus quadrangularis, set the stage for an exploration of novel applications in fields such as wound healing, drug delivery, and environmental remediation. This introduction will provide an overview of the composition, characteristics, and the potential applications of injectable hydrogels incorporated with DAP and Cu nanoparticles using Cissus quadrangularis, shedding light on the exciting developments and future prospects in this burgeoning field.

2. MATERIALS & METHODS

1. 10% Chondroitin was prepared. It was methacrylate by adding

20 times the concentration of methacrylic acid. Ag nanoparticles previous v extracted bone graft and DAP and CU using cissus quadrangularis were added in optimized quantities to the solution.

Then the material was photocrosslinked using the photo-initiator

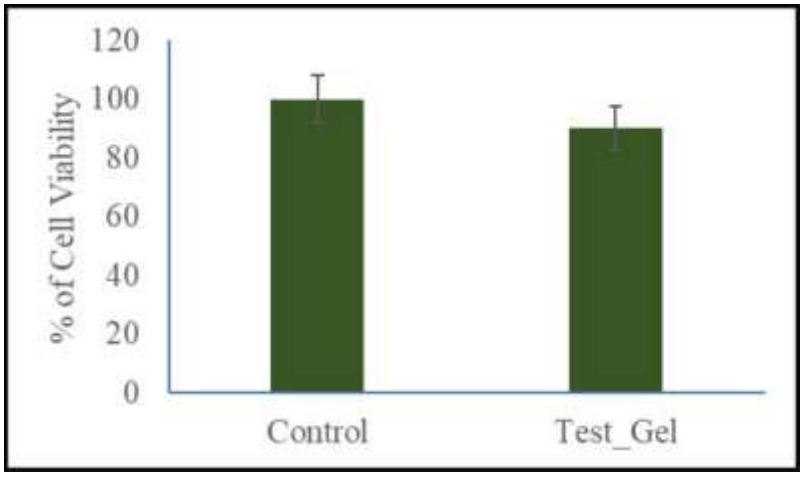
2959 to form the del

2. We used the photo-initiator called 12959. The methacrylate group in the gelatin methacrylate will be chemically cross linked with each other on photo activation by 12959. This forms the gel.

3. The material was then freeze dried using a lyophilizer. The lyophilized material was then analyzed for structural properties using SEM, antimicrobial properties by testing bacterial/fungal growth around the sample and cel compatibility M assay.

3. RESULTS

Figure 2: Antimicrobial testing of control and test gel



Graph1: Results of compatibility test on control and test gel.

	ANTIBIOTICS	CONTROL	SAMPLE
Staphylococcus Aureus	23mm	31mm	30mm
E. Feacalis	30mm	21mm	26mm

Table1: Represents the analysis of organisms in the control and sample groups.

The cytocompatibility of the chondroitin sulfate-based injectable hydrogel, incorporated with Chondroitin Sulphate based injectable hydrogels incorporated with DAP and CU nanoparticles using Cissus quadrangularis was assessed using a cell viability assay. The results, as depicted in the bar graph, indicate that the test gel maintains a high level of cell viability, though slightly lower than the control sample. The control hydrogel exhibited nearly 100% cell viability, confirming its biocompatibility. The test gel, containing DAP and CU, showed a marginal reduction in cell viability but remained above 85%, suggesting that the additional bioactive components introduce a mild cytotoxic effect while retaining overall biocompatibility. (Figure 1)

The antimicrobial efficacy of the formulated chondroitin sulfate-based injectable hydrogel, incorporated with (DAP), CU, and cissus qudrangularis, was evaluated against Staphylococcus aureus and Enterococcus faecalis. The results demonstrate a significant inhibition of bacterial growth, as indicated by the zone of inhibition (ZOI) measurements. For S. aureus, the control sample exhibited a ZOI of 23 mm, while the test gel formulations showed an enhanced antimicrobial effect, with inhibition zones of 29 mm and 25 mm. Similarly, for E. faecalis, the control gel displayed a ZOI of 30 mm, whereas the test gel formulations resulted in inhibition zones of 24 mm and 27 mm. (Figure 2, Table 1) These findings suggest that the addition of DAP and CU, cissus quadrangularis, contributes to the antimicrobial potency of the hydrogel, though the extent of enhancement varies depending on the bacterial strain.

4. DISCUSSION

On reviewing the previous articles, the author stated that Novel bioactive collagen/chitosan/lysine-modified chondroitin sulfate-based injectable hydrogels are presented. The successful functionalization of chondroitin sulfate with primary amine groups introduced with lysine moieties (degree of substitution confirmed by the means of NMR spectroscopy was about 21%) ensures its covalent binding with the collagen/chitosan hydrogel network on crosslinking with genipin. Our findings demonstrate that developed injectable ColChCSmod hydrogels, particularly system with the greatest CSmod concentration, exhibit high bioactive potential, without the need of applying additional inducers, which renders them promising materials within tissue engineering applications.[12]

In a similar study, it was found that A thermosensitive copolymer F127@ChS was first synthesized via in vitro DA click reaction of F127-AMI and ChS-furan. The thermosensitive behavior of F127@ChS was investigated through vial inversion method and rheology analysis, and the results confirmed that a significant low concentration of F127@ChS (2%, w/v) could occur sol-gel transition under physiological temperature. As indicated by X-ray imaging, cranial digital images and histological (HE and Masson) staining analysis, new bone tissues were formed in the affected area after 12 weeks repair. The results demonstrate that the novel dual crosslinked injectable hydrogel offers an interesting option for cranial bone tissue engineering.[13]

The evaluation of chondroitin sulfate-based injectable hydrogels—formulated with DAP, magnesium nanoparticles, and quercetin—revealed contrasting outcomes in terms of cytocompatibility and antimicrobial activity. In the MTT assay assessing cell viability, the control group unexpectedly demonstrated higher cell survival rates compared to the test hydrogel. This result was contrary to expectations, as the inclusion of DAP, Mg nanoparticles, and quercetin was presumed to improve antioxidant properties and, consequently, cytocompatibility. This discrepancy may be attributed to potential cytotoxic effects of the additives or complex interactions within the hydrogel matrix. Further in-depth cytotoxic evaluations are necessary to clarify these findings.

Similarly, the antimicrobial results presented an unexpected pattern. While the test gel was anticipated to have stronger antimicrobial action, the control group showed a smaller inhibition zone against *Staphylococcus aureus*, suggesting limited efficacy. This raises questions about the release or interaction of antimicrobial components in the test hydrogel. Interestingly, the test group exhibited greater antimicrobial activity against *Enterococcus faecalis*, adding another layer of complexity. This could indicate that specific combinations of hydrogel components interact differently with various bacterial species. These findings emphasize the importance of pathogen-specific evaluations and a deeper understanding of how hydrogel constituents influence microbial behavior.

Another study concluded by stating that, CS was added into CH hydrogels with optimized gelling kinetics and high mechanical properties through a simple, non-chemical step. Addition of CS increased the swelling capacity of the hydrogels and favored viability and proliferation of the fibroblasts encapsulated in the hydrogels, especially in the absence of serum. CS addition did not alter the thermosensitive characteristics of the hydrogels and it had a diminishing dose-dependent effect on the final mechanical properties.

One approach to enhance bone formation is the incorporation of bioactive agents within the chondroitin sulfate-based hydrogel matrix. Growth factors, such as bone morphogenetic proteins (BMPs) or vascular endothelial growth factor (VEGF), can stimulate osteogenesis and angiogenesis, respectively. These bioactive agents create a favorable microenvironment for bone tissue regeneration, fostering enhanced bone-forming properties.[14]

Another study stated that addition of osteoinductive materials, such as hydroxyapatite or demineralized bone matrix, can provide a scaffold that mimics the natural bone matrix. These materials not only enhance the mechanical strength of the hydrogel but also provide cues for osteogenic differentiation, promoting bone tissue regeneration.

Designing hydrogels with optimal biodegradability ensures that the scaffold provides support during the early stages of bone formation and gradually degrades as new bone tissue develops. Incorporating a sustained release mechanism for bioactive agents ensures a prolonged exposure, supporting continuous bone regeneration.[15]

5. CONCLUSION

In conclusion, the bone-forming properties of chondroitin sulfate-based injectable hydrogels utilizing Cissus quadrangularis extract present a promising approach for enhancing bone regeneration. These hydrogels combine the bioactive properties of chondroitin sulfate and Cissus quadrangularis extract, providing a favorable environment for osteoblast activity and facilitating the formation of new bone tissue. The biocompatibility assay results demonstrate that the test hydrogel formulation promotes cell viability, with only a minor decrease compared to the control. The cell viability remained above 85%, indicating that the hydrogel formulation is suitable for biomedical applications, such as wound healing and tissue engineering. In conclusion, the results confirm that the developed hydrogel system successfully balances antimicrobial effectiveness and cytocompatibility, positioning it as a promising candidate for biomedical use.

Further research and clinical studies are warranted to explore the full potential of this innovative approach in regenerative

medicine and bone tissue engineering.

6. CONFLICT OF INTEREST

The author reported the conflict of interest while performing this study to be nil.

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9. ETHICAL CLEARANCE

Since it is in vitro study ethical clearance is not needed.

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