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Fabrication and Physico Chemical Characterisation of High Molecular Weight Hyaluronic Acid Based Wound Healing Membrane

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ABSTRACT

Background: Hyaluronic acid, is a natural straight polysaccharide, has attracted researchers attention from its initial detection and isolation from tissues. Because of biocompatibility and a high biodegradation of hyaluronic acid, it tracks down wide application in bioengineering and biomedicine: from biorevitalizing skin beauty care products and endoprosthesis of joint liquid to polymeric platforms and wound dressings. Several biopolymers are utilized for the preparation of bioactive wound dressings, including hyaluronic acid (HA), cellulose, chitin, chitosan, fibrin, alginate, elastin, dextran, collagen, gelatin, etc. These polymers possess excellent properties that can be significantly beneficial in wound healing, such as non-toxicity, biodegradability, biocompatibility, readily available, and non-immunogenicity.

Aims: To evaluate the fabrication and physical chemical characterisation properties of high molecular weight hyaluronic acid.

Materials and Methods: Fabrication of Scaffolds: Test 1 contains the stock solution of 1% HA(H), 0.5% carrageenan and 1% gelatin. To fabricate the scaffold, the materials were blended in the ratio 6:1:3 respectively. Test 2 contains the stock solution of 1% HA(H), 1% carrageenan and 1% gelatin. To fabricate the scaffold, the materials were blended in the ratio 6:1:3, respectively. Then the control group contains 1% of HA(H) and 1% gelatin blended in a ratio of 6:4. 3ml of the homogeneous mixture was transferred to six-well plates. 100 ul of the crosslinking agents TPP (15%) was added to each well. The plates were stored at in-20 C for 12 hrs and followed by-80 C overnight. The samples were then lyophilized for 24hrs and kept in dry condition.

Results: Contact angle of the homogeneous mixture of test 1 and test 2. The contact angle of Test 1 of 0.5% carrageenan with HA(H) is 63.52 and Test 2 of 1% carrageenan with HA(H) includes 70.15. HA plays an essential role in tissue repair by stimulating cell migration, adhesion, and proliferation. Studies demonstrated that HA was incorporated into scaffolds for the treatment of wounds. Several studies on animals that used wound models evaluated the effectiveness of HA-based scaffolds. These biologically derived scaffolds, which support dermal regeneration and wound healing, are used. One instance involved dermal/epidermal Directly trapped cell fractions inside the hydrogel, hastened the healing of wounds.

Conclusions: From the present study, it can be concluded that utilizing GTR related to high molecular weight hyaluronic acid in a regenerative strategy to treat disfigurements provided a sizable extra benefit. Since hydrogels incorporate dynamic amide bonds that can immediately attract proteins for tissue engineering purposes, they can be functionalized to resemble the makeup of an extracellular matrix.

KEYWORDS: Biopolymers, High molecular weight, Hyaluronic acid, Wound healing,

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INTRODUCTION

Hyaluronic acid, is a natural straight polysaccharide, has attracted researchers attention from its initial detection and isolation from tissues. Because of biocompatibility and a high biodegradation of hyaluronic acid, it tracks down wide application in bioengineering and biomedicine: from biorevitalizing skin beauty care products and endoprosthesis of joint liquid to polymeric platforms and wound dressings(1). Be that as it may, the principal properties of fluid polysaccharide arrangements with various subatomic loads are different(2). Additionally, the restorative impact of hyaluronic acid put together arrangements straightforwardly depends with respect to the atomic load of the biopolymer. The relationship between the atomic load of hyaluronic acid and its unique properties. Specific accentuation is put on the underlying, physical and physico-substance properties of hyaluronic acid in water arrangements, as well as their degradability(3).

Skin and delicate tissue wounds, like consume, ulcer or other horrible harms, address a significant medical services issue in the whole world in regards to the outcome of the treatment and the expenses related with it. Although a few injury dressings materials have been created, the issue of wound administration is a long way from being settled. The enormous test of wound treatment remains advancing quicker wound healing and reducing the occurrence of bacterial infection(4). An ideal injury dressing material ought to have explicit necessities, for example, keeping an environment and electrolyte balance at the injury interface, permitting vaporous trade, eliminating overabundance of exudates, having antimicrobial properties for disease control, and advancing quicker wound recuperating. Moreover, it should be effectively accessible, cheap, non-hypersensitive, and ought to likewise have hemostatic and pain relieving properties(5).

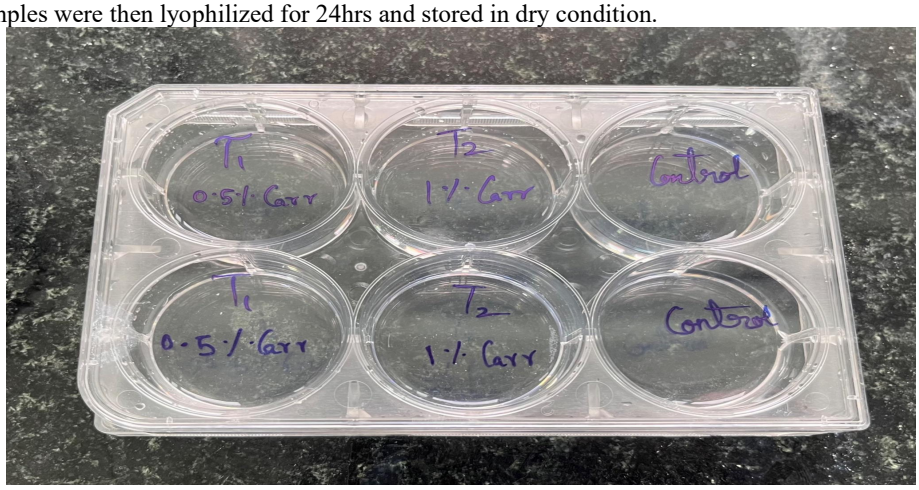
A few biopolymers are used for the readiness of bioactive injury dressings, including hyaluronic acid (HA), cellulose, chitin, chitosan, fibrin, alginate, elastin, dextran, collagen, and gelatin, etc. These polymers have superb properties that can be altogether advantageous in injury recuperating, for example, non-poisonousness, biodegradability, biocompatibility, promptly accessibility, and non-immunogenicity(6). These polymers can be joined with manufactured polymers to work on their mechanical properties. HA is likewise perceived as a potential biomaterial for the improvement of wound dressing(7). There are different reports that stressed the injury mending viability of HA in animating epithelial and mesenchymal cell separation and movement, subsequently further developing collagen statement and angiogenesis. The biodegradation of this injury dressing altogether brings about the arrival of HA that advances epithelial cell expansion(8)

The aim of the present study is to assess the creation and physico chemical characterisation properties of high atomic weight hyaluronic acid.

MATERIALS AND METHODS:

Fabrication of Scaffolds:

Test 1 contains the stock solution of 1% HA(H), 0.5% carrageenan and 1% gelatin. To fabricate the scaffold the materials were blended in the ratio 6:1:3 respectively. Test 2 contains the stock solution of 1% HA(H), 1% carrageenan and 1% gelatin. To fabricate the scaffold the materials were blended in the ratio 6:1:3 respectively. Then the control group contains 1% of HA(H) and 1% gelatin blended in the ratio of 6:4. 3ml of the homogeneous mixture was transferred to six well plates. 100 ul of the crosslinking agents TPP (15%) was added to each well. The plates were stored in-20 C for 12 hrs and followed by-80 C overnight. The samples were then lyophilized for 24hrs and stored in dry condition.



SEM Analysis:

The morphological characteristics of scaffolds were observed using scanning electron microscopy (SEM, JEOL, Tokyo, Japan) after freeze drying. The cross-sections of freeze-dried samples were coated with platinum via a sputter-coater at ambient temperature. Micrographs of all scaffolds were taken at 100X.

Fourier transform infrared (FT-IR) analysis

Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) is a powerful technique to determine any possible chemical interaction ATR-FTIR spectroscopic analysis was performed using Bruker ATR infrared spectrometer (model). The expected pendant functionalities of scaffolds were confirmed by the FT-IR spectrum.

Contact Angle

To determine the hydrophilicity of the scaffolds, water contact angles of the scaffolds were measured by goniometer software. During the measurements, the scaffolds were cut into square specimens with the size of 1 cm × 1 cm, and further they were placed on the testing plate. Subsequently, 50 µL distilled water was carefully dropped onto the prepared specimens. The contact angles between water droplets and the scaffolds were measured by taking photos immediately (within 2s) when the droplets touched the surface of the scaffolds. Three measurements at different positions of each scaffold were conducted.

Swelling ratio (1%) of scaffolds

Swelling/shrinkage studies were performed to calculate the water content (1%) of the scaffolds, wherein 10 mg of freeze-dried scaffolds were placed in 500 µl of PBS at 37 °C. After 24 hours, these scaffolds were removed from the PBS, dabbed with a Kimwipe to remove any excess water on the surface, weighed and placed back into the buffer. The swelling ratio and shrinkage ratio (1%) were calculated using the following equations. All experiments were performed 6 times.

Swelling ratio (SR)= ((Ww−W0)/W0)×100%

W0 and Ww are the initial dry weight and the wet weight, respectively.

Dental Pulp stem cells (hDPSC) Cell Culture:

After obtaining informed consent and ethical approval from SIMATS ethics committee, the Dental Pulp stem cells were isolated from molars. The cells were cultured in DMEM low glucose/10% FBS/1%Penicillin;streptomycin. After two passages, 10000 cells per well were seeded in 48 well plates for cell viability and compatibility assays.

Biocompatibility Analysis (MTT Assay)

100 mg of 5 mm cylindrical blocks were prepared. The prepared blocks were immersed in DMEM- low glucose media formulated with 10 % FBS and 1% Penicillin/streptomycin. The media were collected after 24 hrs and 7 days of immersion and treated with cells to test the compatibility. After 24hrs of culture, add the 10uL/100mL of MTT reagent (5 mg/mL stock) to cultured cells and then incubate for 4 h to allow formation of the formazan dye at 37℃. The medium is exchanged to DMSO (200 µL) and stands for 10min. The reaction product was transferred to a 96 well ELISA plate and A590 was measured with ELISA plate reader.

Statistical analysis

All values are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments. A one-way ANOVA (analysis of variance) was used to test for significant differences, and multiple comparisons were performed using Scheffe's method. Statistical significance was set at $p < 0.05$.

RESULTS

TEST DESCRIPTION	CONTACT ANGLE	ANGLE °
Test 1 - 0.5 %	HA(H) GTR	63.52
Test 2 - 0.1%	HA(H) GTR	70.15

Table 1 : Contact angle of HA(H) homogeneous mixture of Test 1 and Test 2

Table 1 depicts the contact angle of the homogeneous mixture of test 1 and test 2. Contact angle of Test 1 of 0.5% carrageenan with HA(H) contains 63.52 and Test 2 of 1% carrageenan with HA(H) contains 70.15

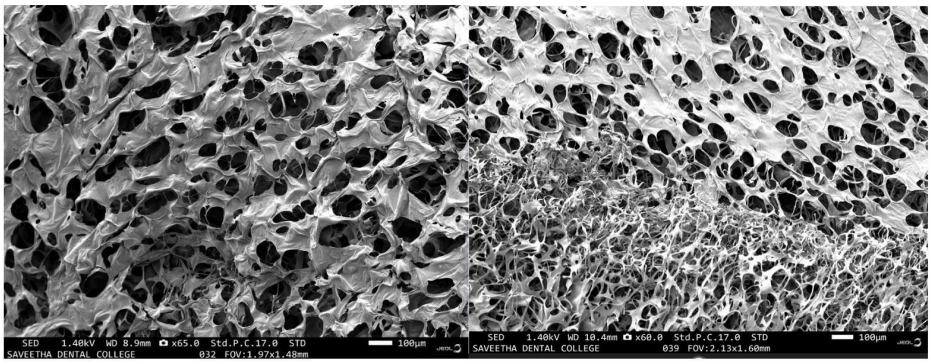


Figure 1: SEM analysis of High molecular weight HA

Figure 1 depicts the Scanning electron microscopy of a high molecular weight GTR membrane with 0.5% carrageenan (left image) and 1% carrageenan (right image).

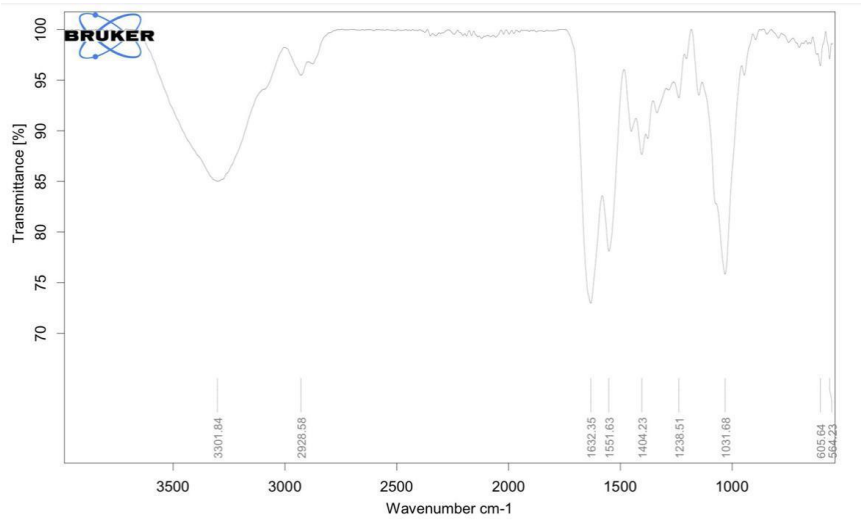


Figure 2: FTIR spectra of high molecular weight HA with 0.5% carrageenan

Figure 2 depicts the FTIR spectra of high molecular weight HA based GTR membrane crosslinked with 0.5% carrageenan

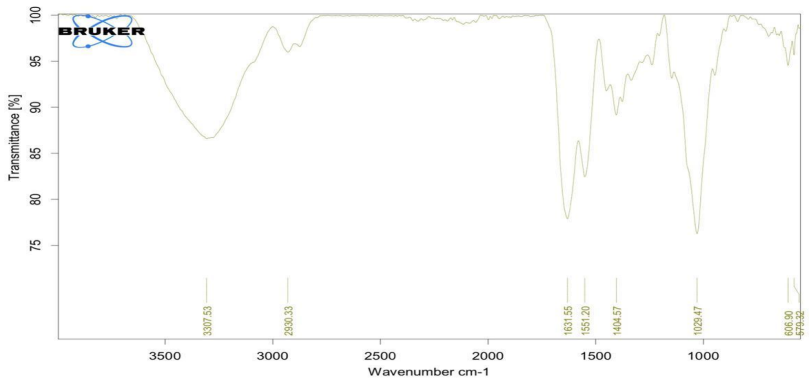


Figure 3: FTIR spectra of high molecular weight HA with 1% carrageenan

Figure 3 depicts the FTIR spectra of high molecular weight HA based GTR membrane crosslinked with 1% carrageenan

DISCUSSION

Formulated gelatin-crosslinked HA-based hydrogels captured with recombinant thrombomodulin for twisted treatment in diabetic mice. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was utilized as the crosslinking specialist that is otherwise called an extensively used water-solvent crosslinking specialist. Fourier-change infrared spectroscopy (FTIR) results affirmed the effective crosslinking between the polymers. The checking electron microscopy (SEM) pictures showed that the hydrogels were permeable (around 20-300 µm in diameter)(9). The pore size of hydrogels was diminished as the grouping of HA expanded. The in vitro water retention examination of hydrogels showed that the water assimilation quickly expanded inside the initial 30 min, and the HA dressings showed more than 11-crease enlarging in one day or less. HA fixation didn't influence the water retention elements of hydrogels. All the HA-based hydrogels displayed great water ingestion conduct, which might assist with retaining wound exudates and advance great medication retention at the injury bed. The in vitro drug discharge energy at 33 °C was fast with more than 40% arrival of thrombomodulin from the HA-based hydrogels in the initial 3 h followed by a supported delivery(10). The total arrival of thrombomodulin from HA hydrogels happened in 12 hrs. The in vivo mending concentrates on diabetic mice showed that the hydrogels stacked with thrombomodulin exhibited a really recuperating impact when contrasted with plain hydrogels however didn't vary from free thrombomodulin solution. In situ collagen-hyaluronic corrosive hydrogels refined with fibroblasts (COS-7) and Human microvascular endothelial cells(HMEC)(6). The enlarging examination of hydrogels showed an expanded and steady expanding limit inside 72 h, which came to 45%. This enlarging effectiveness is valuable for conveying signal variables and supplements between cells refined in the HA hydrogel and cells on the injury, bringing about excitement of the phone outgrowth. The antibacterial viability of hydrogels showed roughly 47% of S. aureus and 55% of E. coli were annihilated by post brooding them with the hydrogels at 37°C for 3 h. COS-7 and HMEC embodied inside these hydrogels showed advanced critical cell expansion. The in vivo recuperating examination utilizing full-thickness twisted in mice showed that injuries treated with the hydrogels showed a mending cycle, which was higher contrasted with the business drug and free collagen hydrogel, HA hydrogel tests in light of the fact that the mix of collagen and HA improved the injury recuperating. In situ HA hydrogels epitomized with plasmid DNA encoding vascular endothelial development factor for the treatment of consumed wounds. The in vivo wound mending investigations of the hydrogels utilizing mice exhibited sped up recuperating on braced consume wounds, explicitly by preventing irritation response and animating microvascular advancement while being biocompatible. Wipes are wound dressings that can retain huge amounts of wound exudates, and they give a clammy climate to the injury due to their high

porosity, enlarging profile, and biodegradability. By and large, wipes are non-cement and require optional injury dressing or wraps/tapes to keep them at the injury site. HA subordinates or different polymers have been utilized in mix with HA to conquer the poor mechanical properties shown by HA-based sponge. Arranged fibrin nanoparticles embodied with vascular endothelial development factor (VEGF) and stacked them in planned chitosan-HA composite wipe for diabetic injury dressing(8). SEM pictures of VEGF stacked fibrin nanoparticles showed circular shape with a size range somewhere in the range of 150 and 180 nm, while DLS examination displayed a mean molecule size of 180 nm with a polydispersity record (PDI) of 0.4 and a normal negative surface charge of 28 mV. The FTIR range showed the normal practical gatherings of biopolymers and affirmed the fruitful embodiment of nanoparticles in the sponges(11) The porosity trial showed that every one of the wipes had a porosity that reaches between 65-75%. The mechanical examination results showed a prolongation at break, which showed the adaptability of HA-based wipes that ran between 10-20%, while the elasticity went somewhere in the range of 0.15 and 0.02 MPa. The adaptability of the platform was further developed by expanding HA fixation demonstrating the way that the wipes can be put on any tissue without breaking(12).

CONCLUSION

In the wake of breaking down the information and considering the constraints of the ongoing examination, it can be said that utilizing GTR related to high molecular weight hyaluronic acid in a regenerative strategy to treat disfigurements provided a sizable extra benefit. Since hydrogels incorporate dynamic amide bonds that can immediately attract proteins for tissue engineering purposes, they can be functionalized to resemble the make-up of an extracellular matrix. Further study is needed to demonstrate the clinical result in the patients.

Conflicts of Interest

The authors declare no conflict of interest.

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