animal. Soft tissue fillers were evaluated separately by subcutaneous injection. In addition, 3D printed scaffolds were combined with the injectable filler prior to implantation to explore superior tissue regenerative potential.
***Results:** *In-vitro* results demonstrated greater cell adhesion and proliferation on rhCollagen-based scaffolds, compared to formulations without rhCollagen. *In vivo* histopathological evaluation showed significant ingrowth of vascularized tissue and fat with minimal fibrosis in all study groups. Addition of the filler to the 3D construct mainly contributed to superior tissue integration of the implant.
***Conclusion/Significance:** The unique biological and physical properties of rhCollagen-based compositions provide the flexibility to formulate versatile fillers and bioinks that are compatible with different regenerative medicine applications such as bioprinting of organs and tissues and soft tissue filling and contouring. Preclinical studies demonstrated excellent biological performance and tissue regenerative potential of the rhCollagen-based scaffolds both *in-vitro* and *in-vivo*. rhCollagen-based formulations can therefore provide a superior solution for regenerative medicine applications and overcome limitations associated with traditional methods.

96 - Tough And 3d Printable Marine Based Hydrogel For Wound Healing Application

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***Purpose/Objectives:** We have a relatively poor toolkit for healing wounds when compared to advanced therapies for various diseases such as cancer. Innovative biomaterials are vital for addressing acute inflammation and antibiotic-resistant bacteria in infected wounds. In addition to the bioactivity of wound healing materials, the construction of wound dressing materials as a carrier of wound healing bioactive compounds plays a significant role. We proposed a facile and safe approach to prepare mechanically reinforced chitosan-based hydrogels via a phenolated polyelectrolyte complex (PHEC) and enzyme-mediated crosslinking, as well as enhanced biological features via incorporating chitooligosaccharides (COS).
***Methodology:** The 3D printable hydrogel was constructed based on phenolated chitosan and alginate (1-3 wt.%) by dropwise addition of phenolated alginate to the phenolated chitosan solution under strong agitation resulted in PHEC formation with high dynamic viscosity. Subsequently, a co-enzymatically mediated crosslinking using horseradish (HRP) (1 U/mL) and glucose oxidase (GOx) (5 U/mL) triggered by glucose (5.5 mM) was performed for hydrogel solidification. The physiochemical and wound healing potential of hydrogel containing COS as a bioagent were comprehensively assessed via in vitro and in vivo analysis.
***Results:** PHEC resulted in the formation of 51in situ phenol-functionalized microfibers leading to an increase in dynamic viscosity (20 times) modulus (60 times), toughness (2-3 times) which paves the way for excellent 3D printability of the hydrogel with high flexibility, and mechanical enhancement. In addition, the Gox-cascade reaction resulted in a gradual release of hydrogen peroxide (H2O2) to activate the HRP, resulting in a mild enzymatic crosslinking of the phenol-containing polysaccharides (chitosan and alginate). Besides, glucono-delta-lactone (GDL), as a by-product of glucose oxidation reaction, was hydrolyzed and gradually donated protons, leading to the protonation of more free amino groups of

chitosan and COS, which resulted in the enhancement of the electrostatic interactions with negatively charged alginate. The hydrogel exhibited a suitable swelling ratio (<50%) and complete biodegradation of hydrogels after nine days, and a heterogeneous microstructure with pore size ranging from 50 to 350 µm. The hydrogel could exhibit flexibility, injectability, moldability, and self-healing property due to the dynamic electrostatic interaction as secondary crosslinking acts as a sacrificial bond for energy dissipation. Biological activities assessment showed that COS incorporation significantly improved the antioxidant (34 to 70 %) and could inhibit the growth of E. coli and S. aureus according to the colony-counting assay. Besides, the hydrogel showed a good 3D cell encapsulation of 3T3L fibroblast with uniform cell spreading and viability. Moreover, in vivo results revealed a significantly higher wound closure after COS incorporation (61.5±3.2 %) compared to control (46.3±3.7 %) at day 14 post-injury re-epithelialization, evidenced by the smaller residual epithelial defects compared with those of PBS-treated (control) wounds after 7 and 14 days of post-injury.
***Conclusion/Significance:** In conclusion, the hydrogel exhibited great potential as a wound dressing hydrogel with high mechanical stability, toughness, self-healing ability, injectability, excellent 3D printability and superior bioactivity to prevent wound infections and speed up skin regeneration. The proposed method offers a green approach for the 3D printing of chitosan-based hydrogels for biomedical applications.

Session Number: 26
Non-invasive Imaging and Analysis of Engineered Tissues
Wednesday, July 13, 2022, 8:00 AM - 9:30 AM

97 - High-frequency Ultrasound To Assess The Acoustic Properties Of Cell-laden Hydrogels In Vitro

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***Purpose/Objectives:** High-frequency ultrasound (US) can measure the acoustic properties of engineered tissues non-invasively, non-destructively, and in real-time with µm-scale resolution. Acoustic properties, including acoustic attenuation, are related to *intrinsic* material properties, including microstructure, density, and elasticity. Previous attempts to measure the acoustic attenuation of hydrogels failed to account for the attenuation of the coupling media and the reflection/transmission of US waves at the coupling media-hydrogel interface and hydrogel-substrate interface. The acoustic attenuation of the coupling media depends on the focal distance of the transducer and the thickness of the hydrogel. Thus, if not accounted for with varying hydrogel thickness, the total attenuation measurement is inaccurate. To address this limitation, we developed an analytical approach that accounts for the frequency-dependent effects of attenuation in coupling media and the reflection/transmission of US waves at the coupling interfaces in cell-seeded hydrogels in vitro.
***Methodology:** We performed 500 µm x 500 µm raster scans of cell-seeded hydrogels with a 40 MHz ultrasound probe to measure pulse echoes focused on the coupling liquid-substrate interface (s_1), sample-substrate interface (s_2), and coupling liquid-sample interface (s_3). In s_1, the transducer was