Collink·3D 50

Collink·3D 50L

Human Collagen BioInk

Collink.3D™ 50 Cat# W10199

Collink.3D™ 50L Cat# W10203

Introduction

Collagen is a structural protein that serves as the basic building block of the extracellular matrix in the human body. Its natural structure, inherent biocompatibility, substantial clinical practice and biodegradability render it an ideal biomaterial for regenerative medicine.

CollPlant has developed Collink.3D™ 50 solution and its lyophilized form Collink.3D™ 50L, the first and only human collagen bioink, based on plant-derived recombinant human type I collagen (rhCollagen) that is mass produced at high consistency.

The product is compatible with major 3D bioprinting technologies and cell types. Collink.3D™ 50 enables scalable and reproducible biofabrication of scaffolds, tissues, and organs for 3D modeling as well as implantation purposes, while ensuring perfect recapitulation of the properties of native tissues and organs. Biofabricated constructs composed of Collink.3D™ 50 offer superior biological performance, consistency and safety.

Description

Collink.3D™ 50 is rhCollagen methcrylamide supplied either in a highly concentrated solution or in a dry form, to be used as a curable bioink platform for biofabrication applications including 3D bioprinting.

RhCollagen, produced from genetically engineered tobacco plants, was chemically modified by converting its free amines, i.e., the α-amino groups of the lysine residues as well as the α-amino groups on the N-termini, to methacrylamides. As a result, approximately 50% of the total primary amines of the collagen molecule were functionalized by methacrylic moieties. Following modification, the protein was further purified and then concentrated to yieldCollink.3D™ 50 in 10 mM HCl solution, and Collink.3D™ 50L powder.

Intended use

Collink.3D™ 50 and Collink.3D™ 50L are intended for the biofabrication of tissue and organ models, 3D cell cultures including spheroids/organoids, as well as scaffolds, tissues, and organs for implantation.

Biofabricated constructs composed of Collink.3D™ 50 can be used in a wide range of tissue model applications including drug discovery, drug screening, disease modeling and tissue testing. In addition, it can be used for the development and manufacture of implantable tissues, scaffolds and organs with complex architectures to meet specific properties.

Collink.3D™ 50 and Collink.3D™ 50L are compatible with major printing technologies, e.g., extrusion, inkjet, photolithography, and laser-induced forward transfer (LIFT).

Collink.3D™ 50 and Collink.3D™ 50L are for R&D use only and are not intended for human use.

Characterization and testing

General

Collink.3D™ 50 is a highly homogenous solution of intact, plant-derived triple-helical human type I collagen, rich in cellular binding domains and free of tissue residues. The product has demonstrated a high safety profile.
Collink.3D™ 50L is a powder form of Collink.3D™ 50, offering additional operational flexibility, specifically in the incorporation into bioink formulations, without limitation of a buffer type or concentration, as well as easy handling.

Table 1: Characteristics of Collink.3D™ 50

Parameter	Specification
pH	1.9 – 2.4
Appearance	Clear, transparent, no visible particles
Identity	Type I collagen
Purity	> 95% Type I collagen
Concentration	13 – 17 mg/ml
Degree of Functionalization#	45-65%
Total Aerobic Microbial Counts	< 10 CFU/ml
Total Yeast and Mold Counts	< 10 CFU/ml
Endotoxins	< 7 EU/ml
Elemental analysis	According to USP<233>
Curing Kinetics- Time to G' >100 Pa	≤ 5 sec

#Determined using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) assay, relatively to the rhCollagen source.

Table 2: Characteristics of Collink.3D™ 50L

Parameter	Specification
pH after reconstitution#	1.9 – 2.4
Color after reconstitution#	Clear, transparent, no visible particles
Appearance: Color	White to Off White
Appearance: Form	Dry cake
Identity	Type I Collagen
Purity	> 95% Type I Collagen
Concentration after reconstitution#	9-11 mg/ml
Degree of Functionalization	45-65 %
Total Aerobic Microbial Counts	< 10 CFU/ml
Total Yeast and Mold Counts	< 10 CFU/ml
Endotoxins	< 7 EU/ml
Elemental Analysis	According to USP<233>
Curing Kinetics- Time to G' >100 Pa	≤ 5 sec
Dry weight	100-120 mg

#For Collink.3D™ 50L 10 mg/mL solution in 10 mM HCl after 3 hours of reconstitution

Structural identity [1]

A representative SDS-PAGE gel image of Collink.3D™ 50 product (Figure 1) shows distinct bands at ~100 kDa corresponding to the characteristic mixture of α1 and α2 chains, with the expected slight shift in the weight of the modified molecules (lanes #3 and #4) vs. the native molecule (lane #2). The absence of low-molecular-weights fractions demonstrates the high protein purity.

Collagen exhibits a characteristic circular dichroism (CD) spectrum with a positive peak at ~220 nm and a negative peak at ~198 nm, indicative of its intrinsic secondary structure and folding properties (Figure 2).

The CD spectra of Collink.3D™ 50 and Collink.3D™ 50L indicate that the chemical modification of the molecules, as well as the lyophilization process, did not result in perturbation of the overall conformation and that rhCollagen preserves its native structure and structural integrity.

The typical ratios of the intensity of the positive peak near 220 nm over the intensity of the negative peak near 198 nm (Rpn) values of the modified products used to determine triple-helix content are 0.116 and consistent with fully folded triple helices.

Mechanical properties [2]

Due to its high concentration, Collink.3D™ 50 product can be easily incorporated to form multicomponent compositions. It can be mixed with other polymers (synthetic, natural) and ECM-based additives to develop formulations with well-controlled mechanical and biological properties. It is designed to provide hydrogel compositions with mechanical and rheological properties that can be controlled by collagen concentration, crosslinking conditions and the nature of the additives.

Collink.3D™ 50 does not form a gel at room temperature, which makes it easy to handle and use. It features a shear-thinning profile (characteristic curve is presented in Figure 3A), that can be controlled by concentration adjustment and additives. Upon reconstitution of the dry form product, Collink.3D™ 50L, a slight increase in the characteristic viscosity is expected, compared to the non-lyophilized product. While Collink.3D™ 50 averaged viscosity is 120 cP, the characteristic viscosity of the reconstituted material within the same conditions is 160 cP. Collink.3D™ 50 maintains the physical and biological properties of its non-modified rhcollagen source while allowing fast crosslinking at various light wavelengths using different photoinitiators.

Figure 3B demonstrates the fast curing capability of a 10 mg/mL solution of Collink.3D™ 50 in 10 mM HCl using 0.1% Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as a photoinitiator. After initially attaining G'> 100 Pa within 2.5 seconds, the material reaches a plateau G' value of 1200, Pa. The reconstituted Collink.3D™ 50L product within the same conditions is expected to present similar curing kinetics and a higher averaged storage modulus of 2,200 Pa (for 10 mg/mL solution in 10 mM HCl).

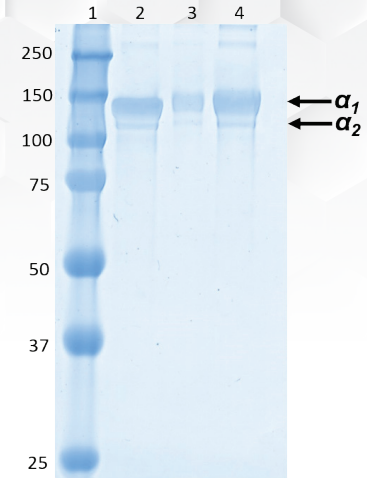

Figure 1: Lane 1: Standard protein marker; Lane 2: Recombinant human type I collagen source for Collink.3D™ 50; Lane 3 and 4: 5 µl and 25 µl sample of Collink.3D™ 50, respectively.

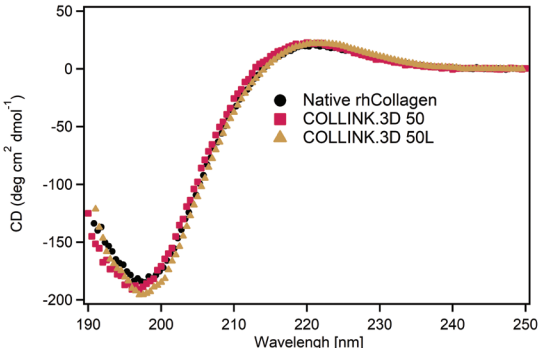

Figure 2: Typical CD spectra of Collink.3D™ 50, Collink.3D™ 50L and the non-modified rhCollagen source at 0.3 mg/mL in a 10 mM HCl solution.

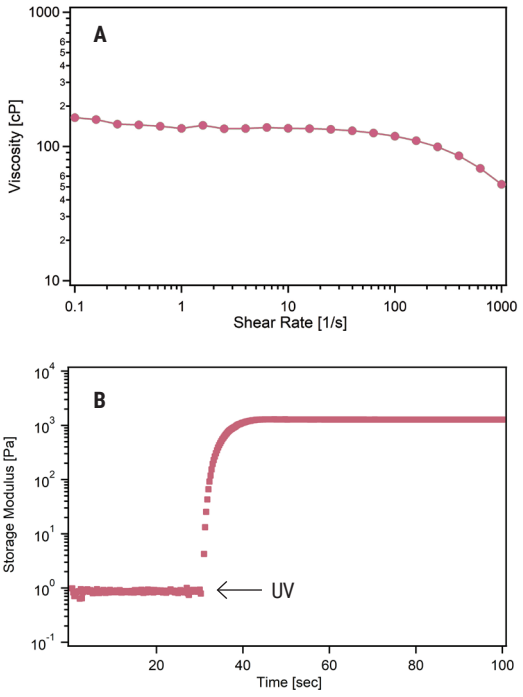

Figure 3: Rheological characterization of a 10 mg/mL Collink.3D™ 50 solution in 10 mM HCl. A: Viscosity measurement using the rotation flow sweep test method. B: Curing kinetics measurement at 365 nm irradiation (50 mW/cm²), using the oscillation time sweep test method and 0.1 % LAP as a photoinitiator.

Figure 4 shows typical gel stiffness measured by applying compression stress on a crosslinked Collink.3D™ 50 discoid-shaped construct, fabricated from a 10 mg/mL solution in 10 mM HCl containing 0.1% 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) as a photoinitiator. The compression curve demonstrates a compression modulus of 26 kPa, with sample failure at 36% strain and 156 kPa stress.

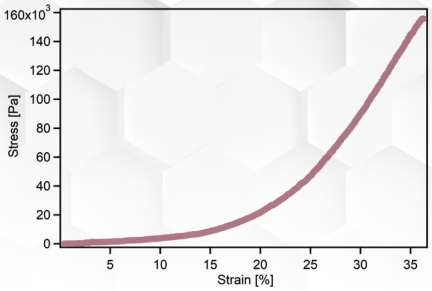

Figure 4: Typical compression stress-strain curve of a disc-shaped Collink.3D™ 50 construct, prepared from 1.6 mL Collink.3D™ 50 exposed to 365 nm irradiation for 2 min.

Cytocompatibility [3]

Collink.3D™ 50-based bioinks are biocompatible materials that enable the biofabrication of constructs with distinct properties that support adhesion, proliferation and function of different cell types and enhance rapid tissue repair. Collink.3D™ 50 can be used for both cell embedding and post-curing cell seeding. The low bioburden renders it compatible with long-term in-vitro studies with no risk of contamination. Formulations based on Collink.3D™ 50 support the culture of various cell types, including fibroblasts, endothelial, epithelial, stem and cancer cells. As shown in Figure 5, cells mixed with Collink.3D™ 50 before printing or seeded onto biofabricated 3D scaffolds, showed superior cell adhesion and proliferation compared to bioink formulations lacking Collink.3D™ 50, with cell viability commonly exceeding 90%. Cells exhibited significantly improved performance, as manifested by cell spreading, well-organized cytoskeletal structures and cell-to-cell interactions. Moreover, Collink.3D™ 50-based bioinks have also been shown to support microvascular network formation and maturation.

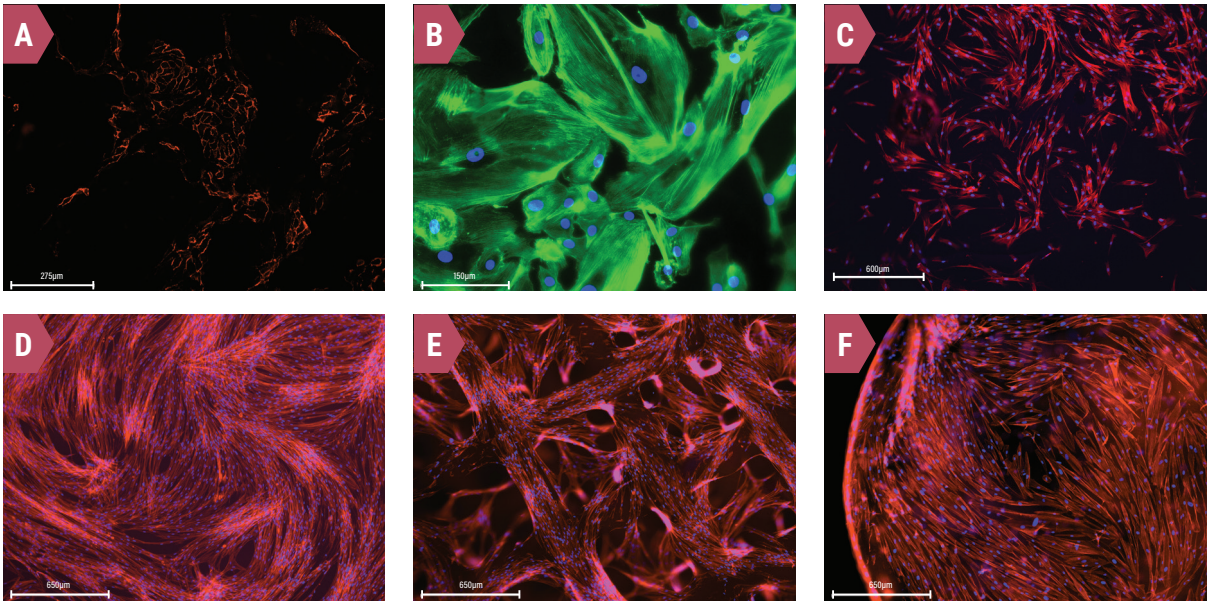

Figure 5: Fluorescent micrographs of cells grown on Collink.3D™ 50 layer [A-C] and 3D-printed constructs [D-F]. CD31 endothelial membrane specific staining of cells after 6 days in culture [A] (10X objective, scale bar: 275 µm). Human endothelial cells stained with DAPI (nuclei) and GFP (actin) after 7 days in culture [B] (20X objective, scale bar: 150 µm). Human fibroblasts stained with DAPI (nuclei) and RFP (actin) after 1 day in culture [C] (4X objective, scale bar: 650 µm). Human fibroblasts proliferated over a 3D-printed disc [D] & mesh [E] after 10 days of incubation (4X objective, scale bar: 650 µm). Spheroid of human fibroblasts embedded in Collink.3D™ 50 after 7 days incubation [F] (4X objective, scale bar: 650 µm). All images were taken with a EVOS 7000 microscope.

Storage and handling

- Collink.3D™ 50 and Collink.3D™ 50L are shipped in a temperature-controlled package. Upon receipt, the products should be stored at 2 °C to 8 °C, protected from light.
- Unless cured, the products should not be frozen or heated above room temperature.
- Avoid heating the products during the curing/printing process.
- Expiration date is indicated in the provided certificate of analysis and is applicable only when the product is handled and stored as directed.
- **For R&D use only. Not intended for human use.**

* (1) (2) (3) Data on File at CollPlant.