Collink·3D 90

Human Collagen BioInk

Cat# W10202

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™ 90, 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™ 90 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 Collink.3D™ 90 offer superior biological performance, consistency and safety.

Description

Collink.3D™ 90 is rhCollagen methacrylamide, 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 90% of the total primary amines of the collagen molecule were converted into methacrylic moieties. Following modification, the protein was further purified and then concentrated to yield the final product in 10 mM HCl solution.

Intended use

Collink.3D™ 90 is 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™ 90 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™ 90 is compatible with major printing technologies, e.g., extrusion, inkjet, photolithography, and laser-induced forward transfer (LIFT).

The Collink.3D™ 90 is for R&D use only and is not intended for human use.

Characterization and testing

General

Collink.3D™ 90 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.

Table 1: Characteristics of Collink.3D™ 90

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#	> 80%
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	≤ 2 sec

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

Structural identity [1]

The SDS-PAGE gel image (Figure 1), comparing Collink.3D™ 90 to additional rhCollagen methacrylamide product Collink.3D™ 50 that is characterized by an averaged DOF of 55%, 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 (Collink.3D™ 50 in lane #3 and Collink.3D™ 90 in lane #5) vs. the native molecules (lanes #2 and #4, respectively). Naturally, the product with higher DOF is characterized by higher Mw, as well as higher molecular weights weak bands of dimeric β-components (~200 kDa) and trimeric γ-components (~300 kDa). The absence of low molecular weight fractions demonstrates the high purity of the proteins.

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 spectrum of Collink.3D™ 90 indicates that the chemical modification of the molecule did not result in perturbation of the overall conformation and that rhCollagen preserves its native structure and structural integrity.

The typical ratio of the intensity of the positive peak near 220 nm over the intensity of the negative peak near 198 nm (Rpn) values used to determine triple-helix content are 0.117 and consistent with fully folded triple helices.

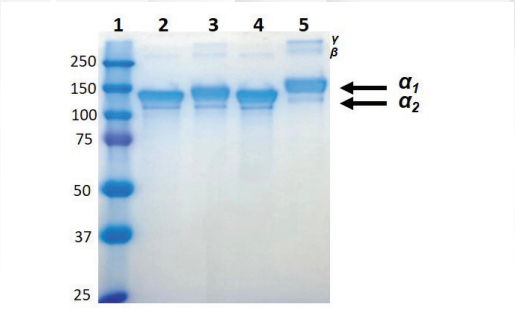

Figure 1: Lane 1: Standard protein marker; Lanes 2,4: Recombinant human type I collagen source for Collink.3D™ products; Lanes 3,5: Collink.3D™ 50 and Collink.3D™ 90, respectively.

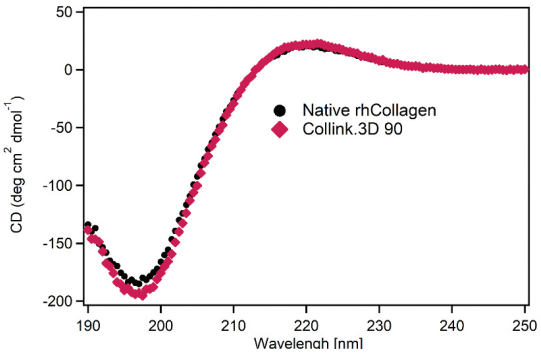

Figure 2: Typical CD spectra of Collink.3D™ 90 (pink) and its rhCollagen source (black) at 0.3 mg/mL in a 10 mM HCl solution.

Mechanical properties [2]

Due to its high concentration, Collink.3D™ 90 product can be easily incorporated to form multicomponent compositions. It can be mixed with other polymers (synthetic, natural) and ECM-based additives to develop compositions 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™ 90 does not form a gel at room temperature, which makes it easy to handle and use. It features a shear-thinning profile that can be controlled by concentration adjustment and additives (characteristic curve for 10 mg/mL solution in 10 mM HCl is presented in Figure 3A), with a typical average viscosity of 180 cP.

Collink.3D™ 90 maintains the physical and biological properties of its non-modified collagen source while allowing crosslinking at various light wavelengths using different photoinitiators. The characteristic high degree of functionalization of Collink.3D™ 90 products results in remarkably fast curing kinetics to reach high G' values. Figure 3B demonstrates the curing capability of a 10 mg/mL solution of Collink.3D™ 90 in 10 mM HCl using 0.1% Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator. After initially attaining G'> 100 Pa within 1.3 seconds, the material reaches a plateau G' value of 16,500 Pa.

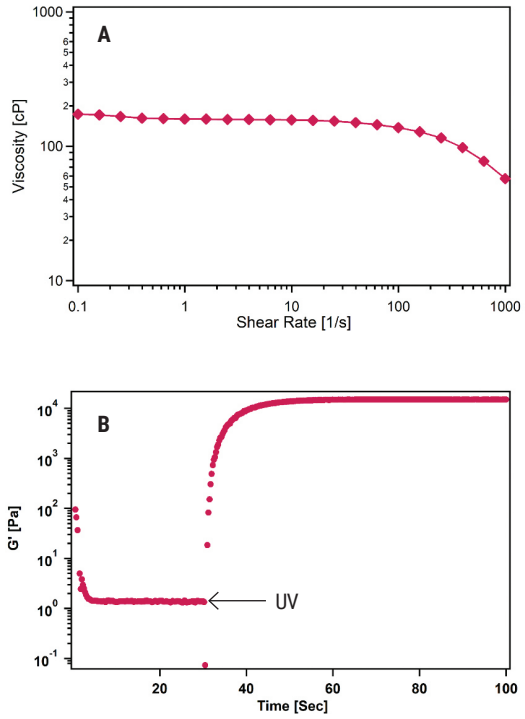

Figure 3: Rheological characterization of a 10 mg/mL Collink.3D™ 90 solution in 10 mM HCl. A: Viscosity measurement using the rotation flow sweep test method. B: Curing kinetics measurement at 365 nm irradiation (50 mW/cm²), using the oscillation time sweep test method and 0.1 % LAP as a photoinitiator.

Moreover, constructs composed of Collink.3D™ 90 present high compression modulus and relatively low strain. Figure 4 shows typical gel stiffness measured by applying compression stress on a crosslinked Collink.3D™ 90 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) photoinitiator. The compression curve demonstrates a compression modulus of 34 kPa, and sample failure at 28% strain.

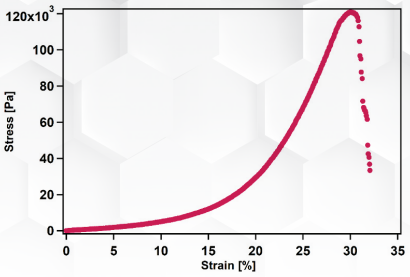

Figure 4: Typical compression stress-strain curve of a disc-shaped Collink.3D™ 90 construct, prepared from 1.6 mL Collink.3D™ 90 exposed to 365 nm irradiation for 2 min.

Cytocompatibility [3]

Collink.3D™ 90-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™ 90 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™ 90 support the culture of various cell types, including fibroblasts, endothelial, epithelial, stem cells and cancer cells. Specifically, the significant fast curing kinetics and the capability to form stiff constructs at low concentrations, make Collink.3D™ 90 ideal for spheroids and organoids biofabrication.

Figure 5A presents an example of a migration study of human fibroblasts into Collink.3D™ 90 crosslinked gel used in standard microfluidic chips at 2.5 mg/mL. The invasion of fibroblasts into Collink.3D™ 90 gel was faster, compared to a chip containing a commercial standard gel within the same concentration. Figure 5B presents vital human fibroblasts embedded in photocured Collink.3D™ 90 at a concentration of 5 mg/mL. Cells spread within the gel and exhibit bridged and elongated actin fibers.

A. Commercial Standard

Collink.3D™ 90

B. Fibroblasts embedded in Collink.3D™ 90 spheroid

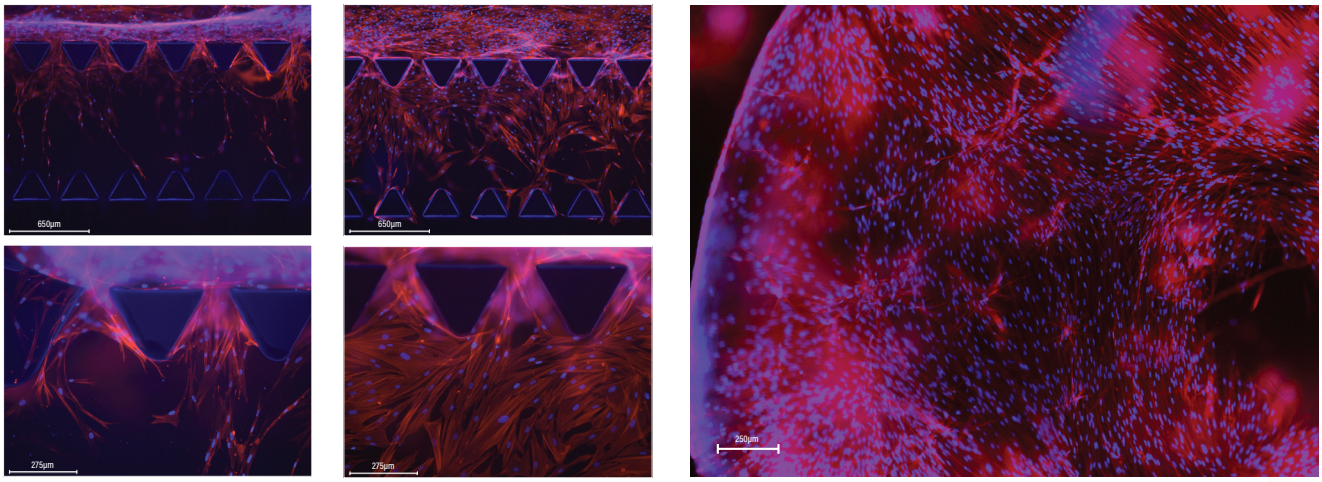

Figure 5: Fluorescent micrographs of 3D cell culture of human fibroblasts within Collink.3D™ 90 hydrogel. Cells are fixated and stained with Hoechst and RFP. All images were taken with a EVOS 7000 microscope. A. Fibroblasts invasion and migration into gel matrices five days after seeding: commercial standard (left) vs. Collink.3D™ 90 (right). B. Fibroblasts embedded in Collink.3D™ 90 spheroid after a week of incubation at 37 °C.

Storage and handling

- Collink.3D™ 90 is shipped in a temperature-controlled package. Upon receipt, the product should be stored at 2 °C to 8 °C, protected from light.
- Unless cured, the product should not be frozen or heated above room temperature.
- Avoid heating the product 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.**

LB0024 REV0

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