PhotoHA®-IRG, Methacrylated Hyaluronic Acid Hydrogel Kit

3D CC Hydrogel

Cat. # CC326

pack size: 1 Kit

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

3D cell culture, including bioprinting, allows for the creation of more physiological cell models by allowing cells to simultaneously interact with integrins on all cell surfaces, resulting in the activation of specific signaling pathways not activated in traditional 2D cell culture methods. Hydrogels are water swollen polymers that allow for the culture of cells in 3-dimensions and can have profound effects on cellular development, differentiation, migration, and function. New areas of tissue engineering such as 3D bioprinting, have utilized UV photocrosslinked methacrylated hydrogel biomaterials (PEGMA, GelMA, HAMA and ColMA etc.) to encapsulate cells to make printable bioinks.

Hyaluronic acid is the most abundant glycosaminoglycan in the body being an important component of several tissues throughout the body. While it is abundant in extracellular matrices, hyaluronan also contributes to tissue hydrodynamics, movement and proliferation of cells, and participates in a number of cell surface receptor interactions. Hyaluronic acid is a polymer of disaccharides, themselves composed of D-glucuronic acid and Nacetyl- D-glucosamine, linked via alternating β-(1→4) and β-(1→3) glycosidic bonds.

The PhotoHA®-IRG, Methacrylated Hyaluronic Acid Hydrogel Kit is based upon purified hyaluronic acid methacrylate (HAMA), which when photocrosslinked provides a native-like 3D environment for cells. In addition to hyaluronic acid methacrylate, the kit includes the photoinitiator Irgacure 2959 for users to easily fine tune their photocrosslinking experiments (i.e. altering hydrogel stiffness or gelling speeds).

Kit Components

The PhotoHA®-IRG, Methacrylated Hyaluronic Acid Hydrogel Kit (CC326) contains:

1) CC326-1 (Store at 2-8°C): Irgacure Photoinitiator, 1 X 100 mg (CS226447).

2) CC326-2 (Store at -20°C): Methacrylated Hyaluronic Acid, 1 X 100 mg (CS226448).

Quality Control

Appearance: Lyophilized Powder
Sterility (USP modified): No Growth
Grafting Efficiency: 50-70%
pH: 6.0-8.0
Osmolality: 200-400 mOsmo H20/kg in 1X PBS
Molecular Weight: 100 – 150 kDa
NMR: Characteristic
Cell Compatibility: Characteristic

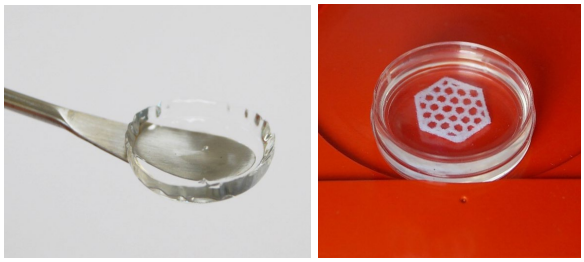

Figure 1. 3D printing of PhotoHA® Methacrylated Hyaluronic Acid Hydrogels can be used as native bioinks for tissue engineering bioprinting applications.

Rev.1.0/2020-04-24/CC326/NJA

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Instructions for Use

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions. Recommended concentrations are 5-30 mg/ml (0.5-3.0%). The following recommended instructions are for a 1% hyaluronic acid (HA) methacrylate solution. Adjustments to this protocol may be required for various concentrations.

1. Add 10 ml of 1X phosphate buffer saline (PBS), water or cell culture media to the 100 mg of lyophilized methacrylated HA powder.
2. Mix on a shaker table or rotator plate until fully solubilized (~30 to 60 minutes) at 2-10°C. *Note: Solubilization times may vary depending on the desired concentration and volume of PBS, water or medium added.*
3. To solubilize the photoinitiator, add 1 mL of neat methanol to the vial of photoinitiator containing 100 mg of Irgacure and vortex. This makes a 10% solution. *Note: The photoinitiator in solution has a shelf life of 2 weeks. Only dissolve required amount of photoinitiator required. Store remaining photoinitiator (powder or solution) at 2 to 10°C.*
4. Calculate the volume of the photoinitiator required by multiplying the total volume of HA methacrylate required by 0.01. For example, if you making 10 ml of HA methacrylate, the calculated volume of the photoinitiator to add is 100 µl.
5. Add the calculated volume of photoinitiator to the required volume of HA methacrylate solution and mix thoroughly.
6. Add your cells to the HA methacrylate/photoinitiator solution.
7. Dispense your HA methacrylate /photoinitiator/cell solution into the desired cultureware (i.e. 6-well plate, 48-well plate).
8. For UV-crosslinking, place the hydrogel printed structure directly under a 365 nm UV light crosslinking source.

Note: Longer exposure allows more crosslinking, though each cell type withstands different degrees of UV light and free radical exposure (generated by the photoinitiator) that mediates crosslinking.

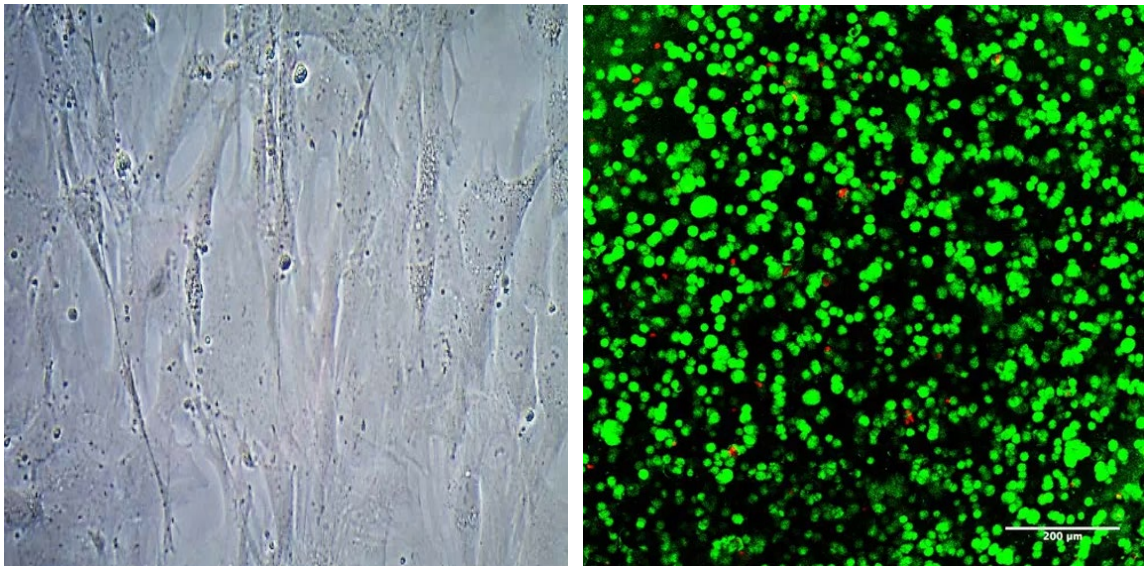

Figure 2. 3D culture of human mesenchymal stem cells using PhotoHA® hydrogels. Human bone marrow mesenchymal stem cells (20 x 10^6cells/mL) were encapsulated in 50 µL 1% PhotoHA® hydrogels (~4.7mm x 2mm). PhotoHA® hydrogels were crosslinked with 0.05% Irgacure 2959 and exposed to 2 mW/cm^2 UV light (320-390 nm) for 10 minutes. After 24 hours, encapsulated cells were stained with live/dead assay (green= viable, red=dead) and subsequently imaged on a Leica SP5 confocal microscope.

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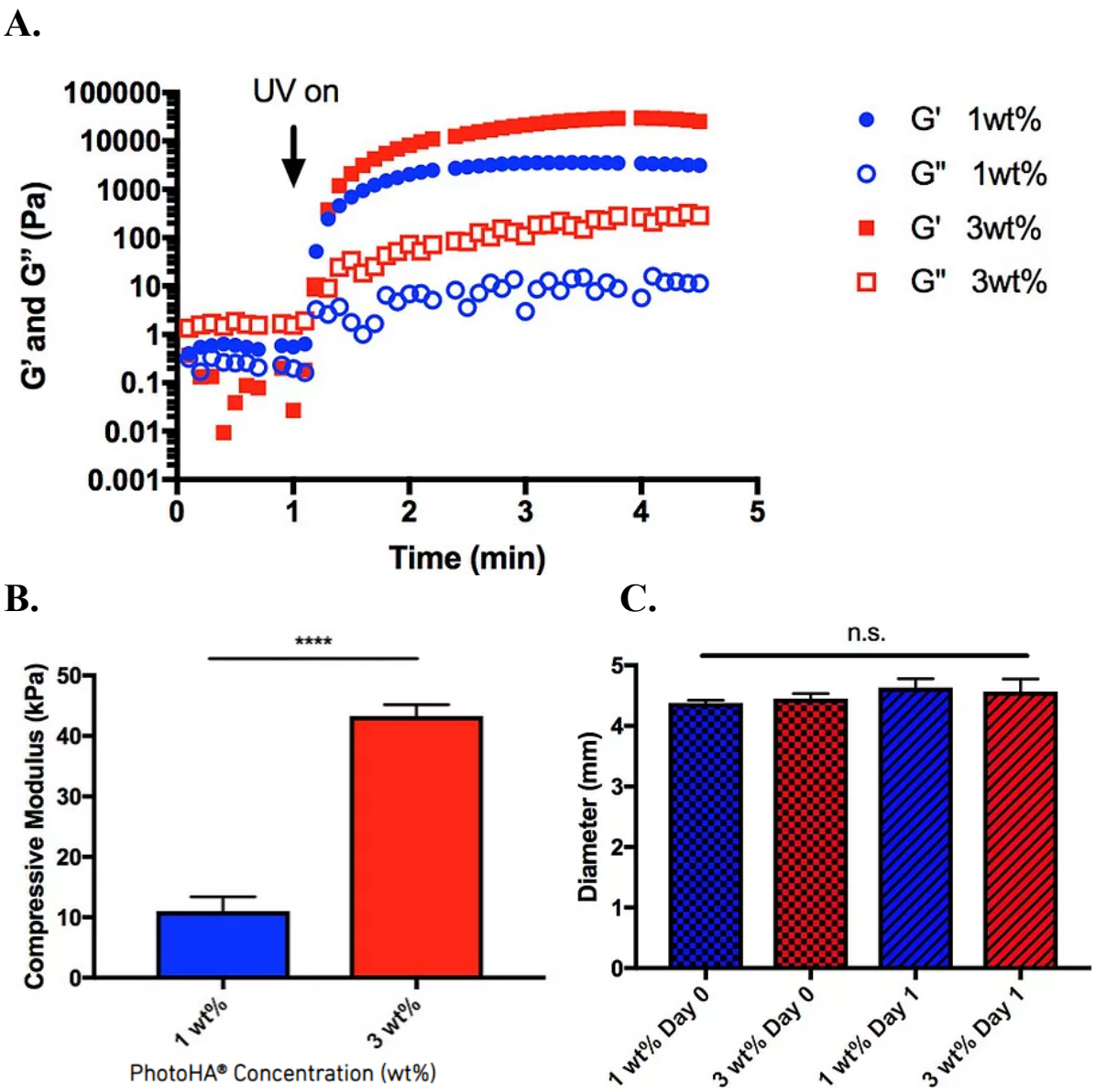

Figure 3. Physical properties of PhotoHA® hydrogels. A) Reaction behavior of PhotoHA®. Rheological time sweeps of PhotoHA® hydrogels crosslinking with exposure to UV light (320-390nm) and in the presence of 0.05% Irgacure 2959. After 1 minute, the macromer solution was exposed to UV light, resulting in the plateau of moduli before 5 minutes. B) Compressive modulus of PhotoHA®. Dynamic mechanical analysis was performed on 50 µL hydrogels of various concentrations (1%/3%). Hydrogels were secured within a fluid cup via a 0.01 N pre-load and compressed to 30% strain at a rate of 0.5 N/min. The Young's modulus of each hydrogel was calculated as the slope of generated stress-strain curves between 10% and 20% strain. C) Swelling characteristics of PhotoHA®. 50 µL hydrogels of various concentrations (1%/3%) were imaged before and after incubation in phosphate buffered saline at 25°C for 24 hours.

PhotoHA® is a registered trademark of Advanced BioMatrix, Inc.

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