Using Cell Collect Products to Harvest Cells from 3D Constructs

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Abstract

Isolating cells from 3D constructs is often necessary for downstream applications like RT-qPCR, immunoblotting and next-generation sequencing. Often, however, this process involves a harsh enzymatic digestion of the surrounding matrix or a mechanical dissociation process that results in low cell viability and yield. To address these issues, matrix dissociation reagents called Cell Collect A and Cell Collect G were developed and optimized to allow for cell recovery without compromising cell viability.

Introduction

Biomaterials like alginate or gelatin have long been attractive for 3D bioprinting because of their high biocompatibility, ease of gelation, shear reversibility and low cost. But cell isolation from these materials often requires tedious processes of mechanical disruption. As an alternative, we have formulated two proprietary ethylenediaminetetraacetic acid (EDTA)-free enzyme-based reagents called Cell Collect A and Cell Collect G that recover cells from alginate- and gelatin-based bioinks, respectively.

Cell Collect A is a proprietary, chemically stable enzymatic solution that digests alginate in temperatures as low as 4°C. It is highly effective when used with alginate-based bioinks that are crosslinked with multivalent ions (e.g., with our $CaCl_2$ Crosslinking Agent). Cell Collect G is a light-sensitive protease that digests gelatin and collagen in temperatures as low as 4°C without compromising cell viability.

At low concentrations, both Cell Collect products partially degrade bioink matrices. They can create channels in printed constructs and reduce the density of fibrous networks. This enables the regulation of matrix stiffness and porosity, which positively influences cell proliferation, migration and biomolecular diffusion in stiff biomaterials.

Furthermore, when bioinks are fully digested, harvested cells can subsequently be used for cell viability analyses, biomarker assays, RNA isolation, protein extraction, Western blots, qPCR or replating. The following study demonstrates the use of Cell Collect A and Cell Collect G in fully degraded alginate- and gelatin-based bioinks for viability and qPCR assessments.

Materials and methods

Cell preparation

Immortalized mesenchymal stem cells (MSCs) and two cancer cell lines (Panc-1 and MDA-MB 231) were purchased from the American Type Culture Collection (ATCC). All cell lines were cultured according to the

supplier's guidelines and passaged every 3 to 4 days. For further evaluation of downstream applications, plasmids were used to genetically label Panc-1 cells with GFP and MDA-MB-231 cells with mCherry. MSCs and Panc-1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Cat #16000044) and 1% penicillin streptomycin (Gibco, Ref #1509-70-063). MDA-MB 231 cells were grown in complete bicarbonate-free Leibovitz L-15 medium with L-glutamine (Corning, Ref #10-045-CV) under 0% CO_2 culture conditions.

Bioink preparation and bioprinting

Up to $5x10^6$ cells of each type were collected and resuspended in 100 µL Hanks' Balanced Salt Solution (HBSS), then mixed with 900 µL GelMA, GelXA or Coll 1. Once homogenized, each bioink was dispensed onto a 24-well plate using a 22G nozzle (CELLINK) and the Droplet Print function on a BIO X (CELLINK). GelMA droplets were photocrosslinked for 10 seconds at 5 cm, Coll 1 droplets were thermally crosslinked for 20 minutes at 37°C, and GelXA droplets were crosslinked with $CaCl_2$ Crosslinking Agent (CELLINK). All cell-laden droplets were incubated in complete growth medium for 5 days at their optimal culture conditions.

Cell Collect treatment and cell harvesting

Cell Collect A (CELLINK, LR0100006001) was diluted in Dulbecco's Phosphate-Buffered Saline (DPBS) to achieve a 1X concentration for droplet digestion, as described in Table 1. Cell Collect G solution was reconstituted in HBSS, per CELLINK protocols, and prepared as a 1X concentration (Table 2). Before recovering cells from 3D constructs, cell culture medium was removed from the desired wells, and prepared Cell Collect solutions were added to each well at a volume of 10:1 µL (Cell Collect Solution: Bioink Droplet). Once submerged, droplet well plates were placed on a cell shaker for approximately 30 minutes, until fully degraded. A gentle trituration with a large orifice pipette tip was applied to fully break down the constructs into a single cell suspension. The final suspension was filtered through a cell strainer and centrifuged at 400 G for 3 to 4 minutes. Resulting cell pellets were kept on ice until further use.

Table 1. *Recommended concentrations of Cell Collect A for full or partial bioink digestion.*

Bioink Volume (µL)	*Degradation*	*Cell Collect A Concentration*	*Digestion Time at 4°C (min)*
10–100	Partial	≤0.5X	15–30
10–25	Full	Original (1X)	15–30
100	Full	Original (1X)	30–60

Table 2. *Recommended concentrations of Cell Collect G for full or partial bioink digestion.*

Bioink Volume (µL)	*Degradation*	*Cell Collect G Concentration*	*Digestion Time at 4°C (min)*
10–25	Partial	≤0.5X	30–45
100	Full	Original (1X)	60–120
400	Full	Original (1X)	120–180

Analysis of cell viability and RNA yield

Cell-laden GelXA or Coll 1 droplets were treated with 1X concentrations of Cell Collect A or Cell Collect G, respectively. Following treatment, cells were collected as described for viability analysis or total RNA isolation. Collected cells were stained with Calcein AM and Propidium Iodide, then cell viability was assessed using an Attune Nxt Flow Cytometer (Thermo Fisher).

For total RNA isolation, Coll 1 and GelXA samples were digested, and RNA was extracted from collected cell pellets using a Zymo Quick RNA Mini Kit. As a control, samples of Coll 1 and GelXA were left untreated and manually homogenized with forceful trituration in cell media and vortexing. The concentration and total yield of the isolated RNA was measured using a Nanodrop (Thermo Fisher) and graphically represented using GraphPad Prism 8.2.1 software.

Statistical analysis

Data are expressed as mean ± standard error (SEM) of two experiments conducted in triplicate. Differences in conditions were analyzed using a one-way analysis of variance (ANOVA) and considered significant for *P* values <0.05.

Results and discussion

Bioink dissociation

The mCherry-labeled MDA-MB 231 cells were bioprinted with GelMA and GelXA and cultured for 5 days before treatment with Cell Collect. Bioprinted constructs were incubated seperately with Cell Collect A or Cell Collect G, and images were acquired throughout treatment. Figure 1 illustrates these constructs at different time points of treatment. Before treatment, the constructs were intact, but after 30 minutes, the cells appeared sedimented with the dissolved matrix. A gentle trituration at the end of treatment allowed for the formation of a single cell suspension.

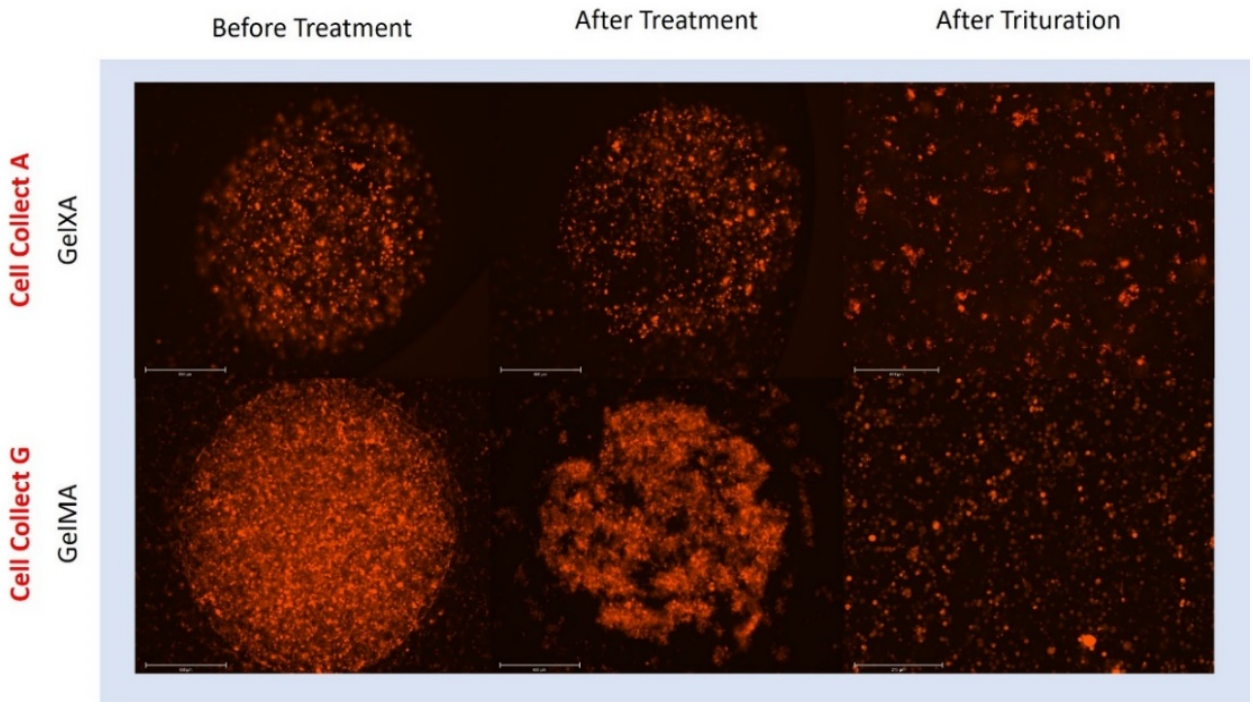

Figure 1. *The mCherry-labeled MDA MB 231 cells in GelMA were bioprinted using the BIO X and cultured for 5 days; they were then treated with Cell Collect G for 30 min to allow for bioink digestion and trituration. Scale bar = 500 µm.*

High cell viability

MSCs harvested from the bioprinted constructs were treated with Cell Collect A and analyzed using flow cytometry technique. At maximum treatment times, Figure 2 shows that treatment with Cell Collect A did not reduce viability compared to the negative control. A cell viability of around 75% was observed following Cell Collect G treatment, suggesting the importance of incubation time and quenching after treatment. A greater viability with Cell Collect G was observed with shorter incubation time (data not shown).

High RNA yield

MDA-MB 231 and Panc-1 cell lines were harvested from the bioprinted constructs and total RNA was isolated using a commercially available kit (Zymo Research). Figure 3 shows that RNA yields from both cell lines were significantly higher for cells harvested using Cell Collect products than for cells that were collected via mechanical dissociation.

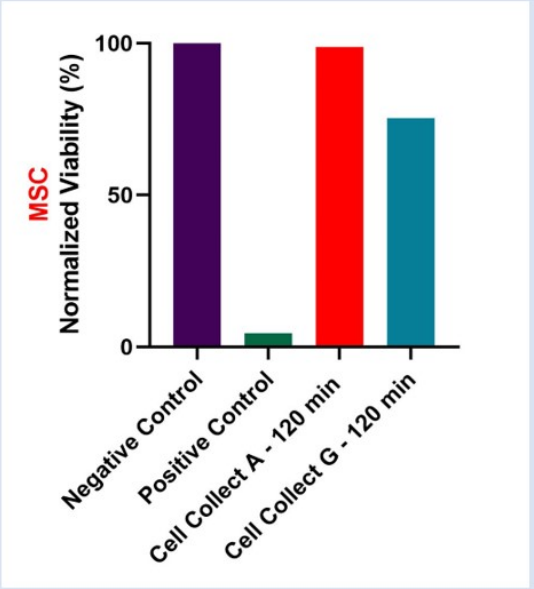

Figure 2. *Cell viability of MSCs treated with Cell Collect A and Cell Collect G for 120 minutes and analyzed with a fluorescence-activated cell sorting (FACS) method.*

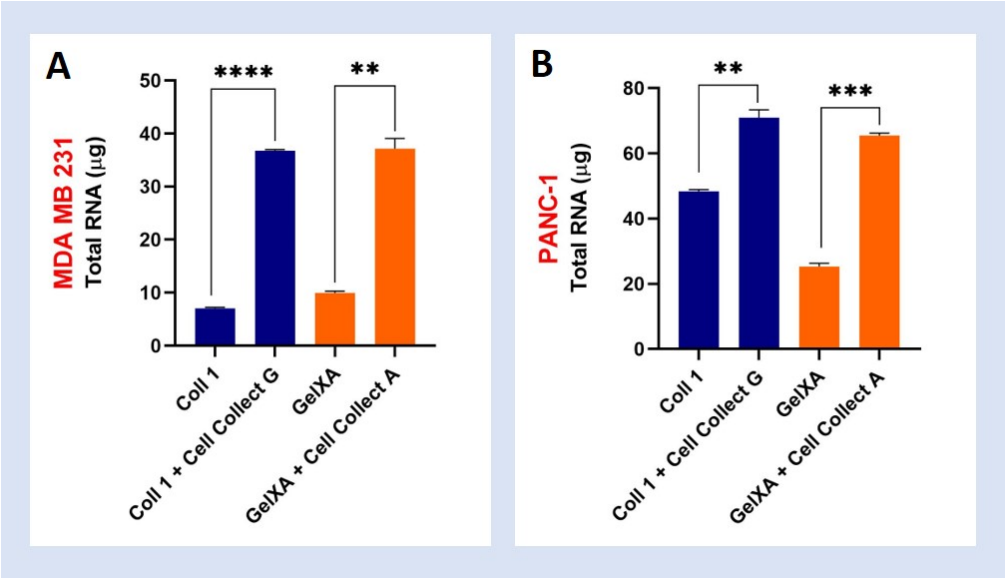

Figure 3. *Total RNA isolated from two different cell lines (Panc-1 and MDA MB 231) bioprinted with GelMA bioink and treated with Cell Collect G in comparison with the same samples mechanically homogenized and lysed. A column-based RNA Isolation Kit was used for the analysis.*

Conclusions

- Cell Collect products allow researchers to recover cells from 3D constructs without compromising their viability for downstream applications, like RNA isolation, protein extraction, single-cell analysis or replating.
- Within 30 minutes, both reagents allow for total or partial bioink digestion, giving users the freedom to regulate ECM stiffness and improve cell proliferation, migration and cell-cell interactions.

References

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