Label-Free Microfluidic Isolation of Intestinal Stem and Progenitor Cells from Native Rat Tissue

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Introduction

Stem and progenitor cells that reside within niches of the intestine are not well understood and characterized. Limited label-free enrichment techniques further confound the ability to perform studies of their differentiation and regenerative potential. This work aims to overcome some of these challenges by the utilization of a novel microfluidic cell isolation technique. The technique relies on affinity-based capture of Lgr5+ and CD133+ cells from rat intestinal crypts.[2] The significance of this method, in comparison with well-established fluorescence- and magnetic bead-based methods, is its label-free nature, which allows rapid capture and elution of target cells, thereby preserving the viability of these highly sensitive cells and making them available for downstream studies ranging from controlled primary cell co-cultures for tissue engineering to more fundamental studies of differentiation.

Materials and Method

Neonatal Lewis rats were sacrificed through decapitation between ages P2 and P5. Intestinal cell fractions were rinsed five times with HBSS and incubated with 20 mL of a 300 U/mL collagense XI and 0.1 mg/mL dispase II. Antibody-coated microfluidic devices (Figure 1a) were fabricated using an alginate hydrogel-based protocol as described by Hatch et al.[1] These devices contain pillar array structures coated with an antibody-functionalized hydrogel which can selectively capture cells; the hydrogel can then be degraded using a chelator to release the bound cells. Antibodies against CD133 and Lgr5 were chosen as the capture molecules. Tissue digestate was injected into the microfluidic devices at a rate of 3 μL/min. Elution of captured target cells was accomplished using 50 mM EDTA at a rate of 10μL/min Eluted cells were embedded in Matrigel™ containing 1μM Jagged-1 and cultured in Advanced DMEM-F12™ supplemented with 50 ng/mL EGF, 100 ng/mL Noggin, and 1 μg/mL R-spondin-1. Culture medium was refreshed every 4 days and growth factors replenished every other day.

Results and Discussion

Implementation of a post array using conjugated anti-CD133/alginate and anti-Lgr5/alginate surfaces yielded a 58% and 49% purity respectively with the injected intestinal cellular populations. A significant enrichment of the CD133 and Lgr5 positive populations was observed in the released elution (Figure 1b,e). Released CD133+ cells were viable and able to produce organoid structures (Figure 1c,d) demonstrating the efficacy of the isolation device. In later culture development, CD133+ positive cells formed a lumen structure containing growing crypt-like domains (Figure 1d).

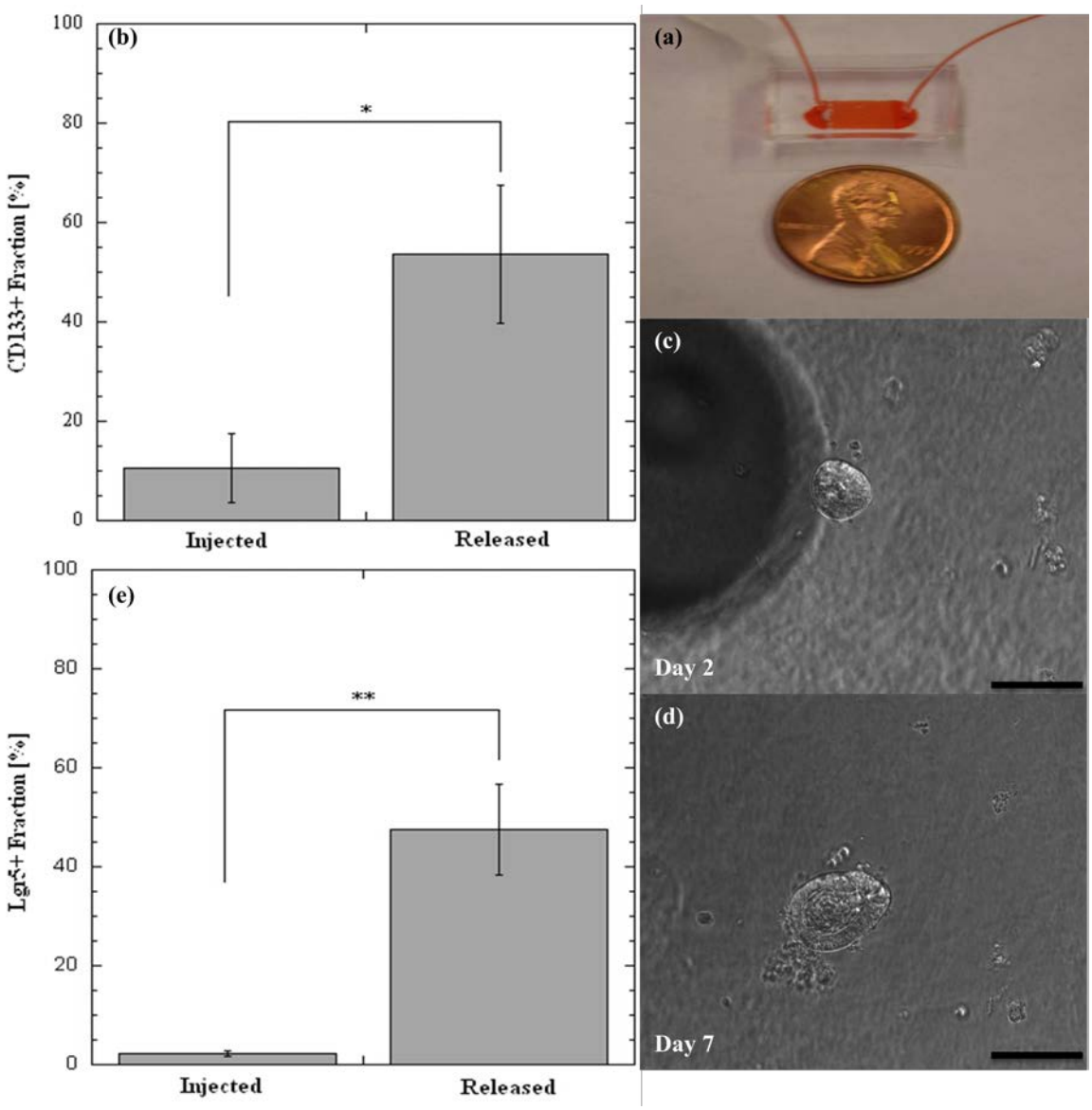

Figure 1 (a) PDMS microfluidic post array. **(b)** Percent purity of anti-CD133/alginate and **(e)** anti-Lgr5/Alginate coated devices, *p<.005; **p<005. **(c)** Early stage organoid formation of released CD133 positive cells embedded in Matrigel™. **(d)** Lumen formation with developing villus and crypt domains at day 7; scale bar=100 μm.

Conclusion

Microfluidic cell separation using antibody functionalized alginate hydrogels are capable isolating CD133+ intestinal progenitor and Lgr5+ intestinal stem cells from tissue with retention of viability, phenotypic identity, and function. The isolated cells have growth characteristics similar to cells isolated using conventional, label-based methods[2] with the added benefit of significantly higher viability. Label free isolation of these cells also makes them attractive candidates for in vivo transplantation.

References

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2.Sato, T.; Vries, R. G.; Snippert, H. J.; van de Wetering, M.; Barker, N.; Stange, D. E.; van Es, J. H.; Abo, A.; Kujala, P.; Peters, P. J.; Clevers, H. *Nature* 2009, *459* (7244), 262-U147.