Application of recombinant collagenase to tissue dissociation and cell recovery

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Abstract

Purpose: Collecting pure and qualified cells from human donor is a critical but challenging process that hinders successful tissue-engineering and cell biology researches. Mostly, collagenase, derived from *Clostridium histolyticum,* is utilized to isolate cells from tissue. However, crude collagenase contains various neutral proteases which can cause cellular damage during tissue dissociation. Therefore, we produced recombinant collagenase from *Escherichia coli* through bioprocessing technology.

Materials and methods: Collagenase potency assay of recombinant collagenase was performed using FALGPA peptide as a substrate (synthetic short polypeptide specifically recognized by collagenase). Based on potency assay, proper unit of recombinant collagenase was treated to lung, kidney and spleen tissue from mice to recover immune cells and bovine cartilage to obtain chondrocytes. Three commercialized enzymes were used as a control representing crude collagenase. To compare cell quality, genetic and CD markers were measured by real time PCR and flow cytometry.

Results: Comparing to control collagenases, immune cells recovered by recombinant collagenase showed fewer numbers, while population of T cell, B cell, NK cell, NKT cell, myeloid and mononuclear phagocyte was similar in both collagenases. However, population of immune cells from spleen obtained by recombinant collagenase showed more even distribution compare to crude collagenase derived ones. Chondrocytes, on the other hand, showed similar recover rate with both cases but ones gained by recombinant collagenase exhibited healthy chondrocyte-like characteristics in regard of genes (*Aggrecan*, *Col1a1*) and surface CD markers (CD14, CD44).

Conclusions: Although tissue dissociation capacity of crude collagenase in lung, kidney and spleen was confirmed to be superior to the recombinant one, distribution of recovered immune cells was mostly similar in both cases. In particular, unlike other two tissues, spleen-originated immune cells with recombinant collagenase showed more even distribution. Since splenic immune cells are used as a reference for immune cells extracted from organs, it is advantageous to present a clear standard using recombinant collagenase. In addition, chondrocytes recovered by using our recombinant collagenase were determined to maintain their original characteristics better than crude collagenase-isolated ones.