Development of particles that simulate a basement membrane surface for use in spinner cell culture

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INTRODUCTION

Most cells, excepting, for example, those in the blood circulation, are attached to components of the extracellular matrix. For many cells, including various types of stem cells, adhesion is to a basement membrane (BM). BMs are unique, highly organised supportive sheet-like structures in the extracellular matrix that are formed at the interface between parenchymal cells and their surrounding tissues. There are different types of BMs in the body; of particular interest are those that act as a tissue boundary where certain cells can attach and those that support very selective differentiation of certain cells, including stem cells.

The BM comprises a range of specific components including type IV collagen, laminin, and various other components including nidogen and heparan sulphate proteoglycans, such as perlecan. Both collagen IV and laminin exist in a range of isoforms, based on different chain compositions that are specific to particular tissues. We, and others, have shown that attaching cells to beads in spinner culture is an excellent method for cell expansion while maintaining phenotype, for example for chondrocytes, and we have developed biodegradable extracellular matrix-based beads using collagen and gelatin to further enhance these attributes. By using biodegradable beads, the cells do not need removal prior to delivery. In the present study we have examined the production of beads based on the BM, including natural and biosynthetic variants.

MATERIALS and METHODS

Natural BM beads/particles: Although there are BMs throughout the body, only in a few cases does the BM contribute a significant proportion of the tissue. We have examined several BM rich tissues as sources to isolate BM, including kidney, lung, placenta and testis. For example, fresh bovine testes were sliced into *c*. 5-10 mm^3 pieces and gently macerated to break the tissue and release cells followed by treatment with pepsin for 1 day. Digested, washed tissue was fragmented using an Ultra Turrax blender (IKA Werke)

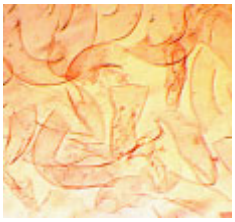
Biosynthetic BM beads: Various cell types, including endothelial cells (derived from human umbilical vein) and Sertoli cells (TM4, ATCC) deposit BM components in culture. Cells were allowed to attach to pre-made supports (eg Cultispher™) for 4 h with intermittent stirring, and then cultured in a continuous spinner culture system with daily addition of sodium ascorbate to 0.05 mg/ml. After 5 d, beads coated with BM were treated with dilute ammonia to detach cells.

Evaluation: The products were examined by microscopy and by immuno-histology for the presence of BM components. Biocompatibility was assessed in cell culture; cells were allowed to attach for 4 h, with intermitted stirring, and then cultured in a continuous spinner culture system for 24 h.

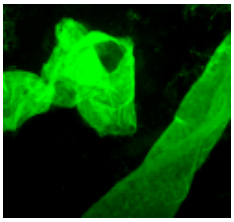
RESULTS

BM particles, prepared as either natural particles or as biosynthetic constructs, both provided particles that were biocompatible and suitable for use in a spinner culture system, where good cell attachment and subsequent growth and amplification was observed using various cell preparations.

Natural BM particles from various tissues produced particles of suitable size, and immuno-histology confirmed the retention of major BM components, including collagen IV and laminin.

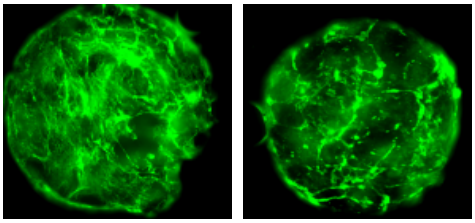


BM tubes prior to fractionation



BM fragments stained for laminin

The size of biosynthetic BM particles could be set by the choice of the supporting bead. Immuno-histology confirmed the deposition of major BM components, including collagen IV and laminin.



Cultispher beads coated with BM by cell culture showing (L) laminin and (R) collagen IV staining.

CONCLUSIONS

The development of natural and biosynthetic BM particles provides natural BM environments for use in cell spinner culture, particularly for stem cell culture. This approach should provide a method for large scale production of stem cells for tissue engineering applications. The choice of tissue or cells type may allow selection of particular BM protein isoforms to allow optimisation of cell growth.