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Identification of the bioactive molecular weight fractions that enhance RPE survival on AMD and aged Bruch's membrane

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Purpose: Although retinal pigment epithelium (RPE) transplants could replace dead or dying RPE in patients with age-related macular degeneration (AMD), RPE transplants (including RPE derived from human embryonic stem cells) performed in patients have showed limited visual improvement. One likely cause of poor outcome following cell replacement therapy in AMD patients is the inability of transplanted RPE to survive on submacular aged and AMD Bruch's membrane (BM). Using an organ culture assay, we showed that conditioned medium harvested from bovine corneal endothelial cells (BCEC-CM) is an effective culture medium for enhanced cell survival on aged and AMD BM. Markedly improved survival was observed after transplanting RPE derived from human embryonic stem cells (~400% improvement), early passage cultured fetal RPE (~900% improvement), and cultured aged adult RPE (~800% improvement). The next step in the development of BCEC-CM as an adjunct to RPE transplant in AMD patients is identification of the bioactive components in BCEC-CM. We identified bioactive fractions of BCEC-CM as a first step towards the identification of the bioactive molecules in BCECCM.

Methods: BCEC-CM was collected from passage-2 BCEC after 72-hour exposure to Madin-Darby Bovine Kidney Maintenance Medium (MDBK-MM). BCEC-CM was fractionated into molecular weight fractions utilizing centrifugal filters (size 3KDa-300KDa). After separation, fractions were tested for bioactivity by analyzing RPE survival on human submacular BM explants established from aged and AMD donor eyes.

Results: Successive removal of high molecular weight components showed no loss of bioactivity with full activity retained in the 50KDa filtrate. Removal of high molecular weight components using the 30KDa and 10KDa filters showed decrease in bioactivity with bioactivity similar to that of CM vehicle only (MDBK-MM) after removal of the molecules above 10KDa. Examination of bioactivity after removal of low molecular weight components showed a complete loss of bioactivity after removal of the 3KDa fraction. Combining the 3KDa filtrate with the 10-50KDa filtrate fully restored bioactivity to that of the 50KDa filtrate and unfractionated BCEC-CM.

Biography

Marco Attilio Eugenio Zarbin, M.D., Ph.D., F.A.C.S. received a B.A. (biochemistry) from Dartmouth College in 1978. Zarbin is an ex officio member of: 1) the board of Governors of the New Jersey Academy of Ophthalmology and 2) the National Advisory Eye Council of the National Institutes of Health. In addition, he is a Member of the American Ophthalmological Society, the Retina Society, the Macula Society, and the Vitreous Society. Zarbin is a Past President of the Board of Trustees of the Association of University Professors of Ophthalmology (AUPO) and has served as the alternate AUPO representative to the Council of the American Academy of Ophthalmology. Zarbin also has served as a member of the Visual Sciences C Study Section, Division of Research/Grants.

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