Synthesis and properties of crosslinked recombinant pro-resilin - an insect rubber-like biomaterial

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Introduction

The design and synthesis of novel biomolecular materials, based on the properties of molecules found in nature, are being mimicked or extended to produce materials with unusual properties. Resilin serves as an energy storage material in insects and facilitates flight, jumping (in fleas and froghoppers) and sound production (cicadas and moths). It is initially produced as a soluble protein and in its mature form is crosslinked via dityrosine units into a very large insoluble polymer. In the present study, we have synthesized a recombinant protein that can be photochemically cross-linked into a resilient rubber-like biomaterial that may be suitable for spinal disc implants.

Materials and Methods

Synthesis of crosslinked recombinant resilin: A portion of exon 1 from the *Drosophila melanogaster* resilin gene (CG15920) (Ardell and Andersen, 2001) was cloned and expressed in *E. coli*. A solution of the recombinant protein was crosslinked using a photochemical method (Elvin *et al*., 2005). The uncrosslinked protein solution and the resulting crosslinked biomaterial were tested *in vitro* for cell viability and toxicity. We are also producing chimeric biomaterials based on resilin and spider silk domains for possible biomaterials applications.

Cell viability assays: Human chondrocytes, isolated from articular cartilage samples at 37°C by standard digestion with trypsin, bacterial collagenase and hyaluronidase, were used a standard live/dead viability assay. All components in the pre-cured resilin were tested for cell cytotoxicity. The polymerised resilin was also assessed for cell attachment and leachables. Cells were tested in the absence or presence of a matrix-supported bead system. Chondrocytes (2.5×10^5 cells) were cultured on Cultispher-S beads (Percell) or gelatin beads in 50 ml DMEM/10% FBS containing 100µg/ml penicillin and streptomycin (culture media) at 37°C with 5% CO_2 in 125 ml spinner bottles. In addition cells were mixed with the 2 part resilin and then cured with blue light. Cells were assessed for viability

Results

The real advantage using resilin over elastin is the ability to cast it rapidly into myriad shapes via the formation of dityrosine crosslinks by visible light in the presence of Ru(Bpy)3 (Figure 1).

In vitro cytotoxicity of resilin in cell culture showed no cell killing. All three components at the concentrations needed for effective curing of the soluble recombinant resilin did not result in any cytotoxicity against primary human chondrocytes that had been isolated from articular cartilage during autologous chondrocyte implantation. Figure 2 shows live/dead viability assay where Calcein AM and Ethidium homodimer-1 (EthD-1) are used to distinguish live and dead cells respectively.

Resilin, when cured and used as a direct substrate, did not support cell attachment and cell proliferation. However, when cells were presented in or on a matrix of gelatin or collagen beads, then cell viability and proliferation was not compromised in the presence of the cured resilin. The cells remain on the matrix and with time migrated out onto the underlying resilin as is the case with TCP.

The ability of cells to remain viable during the curing of the resilin was also evaluated using human chondrocytes cultured on gelatin or collagen beads. In this instance the cells on beads are encapsulated within the resilin polymer. Viability was assessed immediately after curing, within 24 hrs, and after 7 days in culture. Live/dead assessments were made by sectioning the cured resilin and examining various sections for cells on beads. Cells remained viable on the beads and proliferated. In certain areas near the surface quite a lot of cells appear and in other areas cells migrate away from the bead surface, as new matrix is being synthesised.

Conclusions

A range of cell-based studies indicate that the recombinant protein and the components involved in the crosslinking process are non-cytotoxic.

References

Christopher M. Elvin, Andrew G. Carr, Mickey G. Huson, Jane M. Maxwell, Roger D. Pearson, Tony Vuocolo, Nancy E. Liyou, Darren C.C. Wong, David J. Merritt and Nicholas E. Dixon (2005) Synthesis and properties of crosslinked recombinant pro-resilin. Nature. 437(7061):999-1002.

Ardell DH and Andersen SO. (2001) Tentative identification of a resilin gene in Drosophila melanogaster.Insect Biochem Mol Biol. 31(10):965-70.