Nanomedicine & Biotherapeutic Discovery

Extended Abstract

Ultrabithorax-based Materials

Kenith Meissner

Abstract

Ultrabithorax (Ubx) is a Drosophila melanogaster transcription factor protein the the Bondos group discovered has the ability to form ordered materials in vitro. Ubx monomers are produced in E.coli and, following purification, are suspended in a buffer solution and where they do not aggregate in the volume of the solution when refrigerated. When allowed to rest at room temperature, the monomer self assembles at the air/water interface through nucleation, fibril formation and, eventually, film integration. The the self assembled film can then be pulled into a fibre with diameters in the range of 2???50 ?m or lifted off as a film with microscale thickness. These materials are highly elastic and maintain physical properties through cycles of drying and re-hydrating. Novel functions can be directly incorporated into Ubx-based materials via gene fusion to produce chimeric polypeptides capable of both self-assembly and the desired chemical reactivity. Unlike most protein-based materials, the gentle conditions under which Ubx self-assembles enable incorporation of active heterologous proteins. This talk will review recent work on the continued development of this unique materials system including mechanical properties enabled by dityrosine bonding between monomers, dynamics of surface film assembly, and advances in Ubxbased materials production. A key advantage of protein-based materials is the potential to directly incorporate novel functions via gene fusion to produce a single chimeric polypeptide capable of both self-assembly and the desired activity. However, facile production of functionalized protein materials is frequently hampered by the need to trigger materials assembly using conditions that will not irreversibly damage the functional protein. In contrast, the recombinant Drosophila melanogaster transcription factor Ultrabithorax (Ubx) rapidly self-assembles under mild, aqueous conditions to form highly extensible materials with a variety of morphologies. Here, it is demonstrated that materials composed of Ubx chimeras with Enhanced Green Fluorescent Protein (EGFP), mCherry, luciferase, or myoglobin display the functions of the appended proteins, indicating that these activities are neither impaired by the assembly process nor by confinement within the materials.

The Ultrabithorax sequence contains two regions capable of generating materials, only one of which contains motifs found in elastomeric proteins. However, both minimal regions must be included to produce robust materials. Relative to other protein-based materials, Ultrabithorax assembles at significantly reduced concentrations, on faster timescales, and under gentler conditions, properties that facilitate future materials engineering and functionalization. Finally, methods are established that combine EGFP-Ubx and mCherry-Ubx monomers to self-assemble materials patterned on the microscale to macroscale. The self-adhesive properties of Ubx materials also permit manual construction and patterning of more complex forms. The ability to easily functionalize and pattern protein-based materials greatly expands their potential utility in a wide variety of applications.

Although the in vivo function of the Drosophila melanogaster Hox protein Ultrabithorax (Ubx) is to regulate transcription, in vitro Ubx hierarchically self-assembles to form nanoscale to macroscale materials. The morphology, mechanical properties, and functionality (via protein chimeras) of Ubx materials are all easily engineered. Ubx materials are also compatible with cells in culture. These properties make Ubx attractive as a potential tissue engineering scaffold, but to be used as such they must be biocompatible and nonimmunogenic. In this study, we assess whether Ubx materials are suitable for in vivo applications. When implanted into mice, Ubx fibers attracted few immune cells to the implant area. Sera from mice implanted with Ubx contain little to no antibodies capable of recognizing Ubx. Furthermore, Ubx fibers cultured with macrophages in vitro did not lyse or activate the macrophages, as measured by TNF-α and NO secretion. Finally, Ubx fibers do not cause hemolysis when incubated with human red blood cells. The minimal effects observed are comparable with those induced by biomaterials used successfully in vivo. We conclude Ubx materials are biocompatible and nonimmunogenic.

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Kenith Meissner
Swansea University, UK, E-mail: k.meissner@swansea.ac.uk