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Physico-chemical modification of kafirin microstructures for application as biomaterials

By

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DECLARATION

I hereby declare that this thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other University or Institution of Higher Education.

Joseph Ochieng' Anyango
August 2012

ABSTRACT

Physico-chemical modification of kafirin microstructures for application as biomaterials

Joseph Ochieng' Anyango

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Co-supervisor: Dr Janet Taylor

Co-supervisor: Prof Vinny Naidoo

Microparticles produced from kafirin, the sorghum grain prolamin protein, by molecular self-assembly using coacervation with acetic acid solvent are vacuolated. They have shown considerable potential for encapsulation of antioxidants and for preparation of high quality free-standing bioplastic films. However, the functional quality of these kafirin microstructures needs to be improved to exploit their potential application, particularly as biomaterials.

Wet heat, transglutaminase and glutaraldehyde treatments were used to modify the physical structure and chemical properties of the kafirin microstructures. Heat treatment (50–96°C) increased microparticle average size by up to four-fold to ≈20 μm, probably due to disulphide cross-linking of kafirin proteins. The vacuoles within these microparticles enlarged up to >10-fold, probably due to greater expansion of air within the microparticles with higher temperature, as the vacuoles are probably footprints of air bubbles. As with heat treatment, glutaraldehyde (10–30%) treatment resulted in oval microparticles, up to about four-fold larger than the control, probably due to covalent glutaraldehyde-polypeptide linkage. Transglutaminase (0.1–0.6%) treatment had only slight effect on the size and shape of microparticles, probably because kafirin has very low lysine content, inhibiting transglutaminase-catalysed cross-linking through ε-(-glutamyl)-lysine bonding. Surface morphology using atomic force microscopy indicated that the microparticles apparently comprised coalesced nanostructures. With heat and transglutaminase treatments, the microparticles seemed to be composed of round nanostructures that coalesced into random irregular shapes, indicative of non-linear protein aggregation. In contrast, with glutaraldehyde treatment, the nanostructures were spindle-shaped and had a unidirectional orientation,

probably due to linear alignment of the nanostructures controlled by glutaraldehyde-polypeptide linkage.

Thin (<50 µm) films prepared from kafirin microparticles and conventional cast kafirin films were compared in terms of their water stability and other related properties. Films cast from microparticles were more water-stable compared to conventional kafirin films, probably because the large vacuoles within the kafirin microparticles may have enhanced protein solubility in the casting solution, thereby improving the film matrix cohesion. The films prepared from microparticles treated with glutaraldehyde were more water-stable compared to the control, despite the loss of plasticizer, probably due to formation of the covalent glutaraldehyde-polypeptide linkages.

The potential of modified kafirin microparticles to bind bone morphogenetic protein-2 (BMP-2) was investigated. Compared to a collagen standard, the BMP-2 binding capacity of control, heat-treated, transglutaminase-treated and glutaraldehyde-treated kafirin microparticles were 7%, 18%, 34% and 22% higher, respectively, probably mainly due to the vacuoles within the microparticles creating greater binding surface area. The safety, biodegradability and effectiveness of kafirin microparticle film and kafirin microparticle film-BMP-2 system in inducing bone growth were determined by a subcutaneous bioassay using a rat model. Kafirin microparticle film and kafirin microparticle film-BMP-2 system was non-irritant to the animals, probably because kafirin is non-allergenic. The kafirin microparticle film implants showed signs of some degradation but a large proportion of these implants was still intact by Day 28 post implantation, probably because of the low susceptibility of kafirin to mammalian proteolytic enzymes. Kafirin microparticle film-BMP-2 system did not induce bone growth, probably mainly due to low BMP-2 dosage and short study duration.

Modification of kafirin microparticles by wet heat or glutaraldehyde treatment both result in increased size of the microparticles with similar gross structure. However, it is apparent that with both treatments the proteins within the pre-formed kafirin microparticles undergo some form of further assisted-assembly through different mechanisms. It seems that heat-induced disulphide cross-linking reinforces a layer around the nanostructures, probably rich in γ-kafirin polypeptides, that stabilizes the structure of the nanostructures. In contrast, glutaraldehyde-treatment appears to destabilize this structure-stabilizing layer through formation of γ-kafirin polypeptide-glutaraldehyde covalent bonding. This probably offsets the balance of attractive and repulsive forces between the different kafirin subclasses within the

nanostructures, thereby resulting in collapsed nanostructures and linear realignment. A deeper understanding of the mechanism of kafirin self-assembly will be important for further development of kafirin microstructures for different applications.

DEDICATION

This thesis is dedicated to:

My lovely wife Carol,

Daughters Natalie and Hope,

And son Brad

For their love, patience and unwavering support

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