Poster 2.4.06

Incorporation of *Equisetum arvense* extract into silk fibroin–hyaluronic acid sponge matrices for wound dressing applications

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Equisetum arvense extract has frequently been investigated for its antioxidant capacity, antimicrobial and antitumor properties. However, one of the challenges in natural compound investigations is to maintain the stability of polyphenolic compounds during their storage and applications. For this purpose extracts can be incorporated into different forms of biomaterial. In this research silk fibroin (SF) and hyaluronic acid (HA) was used as sponge matrices for wound dressing application. SF is well known biopolymer for its biodegradability, biocompatibility and low inflammation risk properties. *Equisetum arvense* extract was used because of its high polyphenolic content. Incorporation of *E. arvense* extract into SF–HA sponge matrix was performed. By changing the parameters during sponge preparation, different biomaterials in terms of their biodegradability, release properties were obtained. Morphological changes in the sponge-extract biomaterial were investigated with SEM. It was seen that as the hyaluronic acid concentration increased, both the smoothness of the surface and tensile strength were increased. Also by increasing hyaluronic acid content, sponge pore size was increased. Biodegradability and release properties of sponge-extract biomaterial were also investigated. The water take-up of fibroin/HA sponges increased significantly with the increase of HA content. However when plant extract was incorporated into sponges, mechanical strength decreased. The material became more fragile and soluble as the concentration of plant extract increased. This will occur as a result of interaction of phenolic compounds with proteins. Phenolic compounds precipitated the fibroin and this phenomenon resulted in morphological change. Release properties were also changed as a result.

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Poster 2.4.07

Cloning, expression and purification of a single chain variable fragment antibody against Her2 in *Escherichia coli*

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Backgrounds and aims: Single chain variable fragment (ScFv) antibodies are becoming popular therapeutic substitutes to full length monoclonal antibodies. Since ScFv molecules are non-glycosylated, prokaryotic host such as *Escherichia coli* can be used for their expression. Here we evaluated expression of ScFv containing a binding site for domain II of HER2 receptor in *E. coli*.

Methods: The ScFv gene was cloned into the pET22b expression vector. After transformation of competent *E. coli* BL21 (DE3) cells with recombinant plasmid, expression was induced using IPTG. The expressed ScFv was confirmed by SDS-PAGE and western blotting analysis. A Ni-NTA affinity column was used to purifying expressed ScFv.

Results: Molecular weight of ScFv was estimated to be approximately 27 kDa, which confirmed by SDS-PAGE and western blotting assay. ScFv expressed as an insoluble protein which can be highly purified.

Conclusion: Using this expression system, we can obtain high level cytoplasmic expression of ScFv. This ScFv may be a potential candidate to targeting tumor cell overexpressing HER2 receptor.

Keywords: ScFv; *Escherichia coli*; HER2

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Poster 2.4.08

Chemiluminescence in quantitative diagnosis of human breast and prostate tumours

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Chemiluminescense (CL) despite the nonspecific methods that are currently used for both diagnosis and monitoring of cancers provides quantitative results. In this study CL was applied to quantify in immunohistochemistry the HER-2 protein in human mammary invasive ductal carcinoma (IDC) and in lectin histochemistry for *Concanavalin A* (Con A), *Ulex europeus* agglutinin (UEA-I) and *Peanut* agglutinin (PNA) for the evaluation of carbohydrate expression in benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma (PAc). In both method the probes, antibody anti-HER-2 and lectins, were conjugated to acridinium ester (AE) and results were expressed in Relative Light Units (RLU). In IHC for HER-2 in IDC showed different HER-2 expression profiles regarding malignancy levels of this tumour: level 3+ cases presented the highest RLU ($246{,}982 \times 10^3 \pm 2.061 \times 10^3$) compared to 2+ cases ($76{,}146 \times 10^3 \pm 0.290 \times 10^3$), negative ($27{,}415 \times 10^3 \pm 1.445 \times 10^3$) and normal tissues ($27{,}064 \pm 2.060 \times 10^3 \times 10^3$). Prostate tissues showed a lower content of glucose/mannose residues (BPH: 226.931 ± 17.436; PCa: 239.520 ±12.398) and Gal-β(1–3)-GalNAc residues (BPH: 28.754 ± 2.157; PCa: 16.728 ± 1.204) than normal tissues (367.566 ± 48.550 and 409.289 ± 22.336, respectively). However, the highest α-L-fucose expression was observed in PAc (251.118 ± 14.193). Results indicate the chemiluminescent histochemistry as an efficient tool for quantitative differentiation of prostate and breast tissues decreasing the subjectivity in diagnosis which can leads to a differential therapeutic approach.

Keywords: Immunohistochemiluminescence; Mammary tumour; Prostate tumour.

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