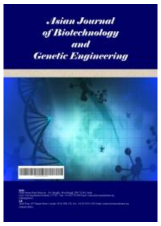

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Application of Engineered Mussel Foot Protein (MFP) from Some Bivalve Species on Cell and Tissue Culture: Mini Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors performed the literature study and manuscript writing. Author RP design the story outline. Authors RP and AA read and approved the final manuscript. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Several species of shellfish from the Bivalve class have unique adaptability to attach their self to a substrate as an effort of defense in strong currents. This is possible by the presence of a special protein which able to coordinate strongly with the substrate even underwater, called Mussel Foot Protein (MFP). MFP is a protein composed of 85 decapeptides where the tyrosine residue group undergoes a process to post-translational become 3,4-dihydroxyphenilalanine or DOPA. The development of research that has been carried out has successfully used MFP in various fields including cell and tissue culture. The use of MFP as a matrix in the culture of several cells and tissues showed an increase in cell quality, cell adhesion and cell growth rate. The existence of several optimizations such as adjustment to optimum environmental conditions and the addition of other additives that support the culture process have also been reported. This article will discuss the latest developments related to MFP application in cell and tissue culture along with the optimization efforts that have been reported. Opportunities for future research development related to MFP applications, especially in the field of biotechnology, will also be discussed accordingly.

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1. INTRODUCTION

Bivalve is a class of organism from the molluscs which are characterized by the presence of a pair of shells (Bivalve means two shells). Generally, this class includes groups of shellfish such as mussels, oysters and clams. Several species in this group have long been known to have important ecological functions in aquatic ecosystems [1]. Especially in terms of water quality, because of its nature which mostly lives as a filter feeder and suspension feeder [2,3]. Their ability to withstand polluted water conditions makes several species also used as bioindicators of water quality [4]. In addition, several species have important economic values and become commercial fishery commodities with high selling value [5]. Apart from being a source of high protein such as green clams and abalone, shells and pearls produced by bivalve species are also used as jewelry with high economic potential [6].

Several species of bivalves have unique strategies for survival in the harsh marine environment. For example, blue clams or *Mytilus edulis*, which are known to stick firmly to hard substrates so they can tackle strong ocean waves. They are known to produce hundreds of fine threads, 4 to 5 cm in length, that are bonded to one another called byssus [7]. The byssus is coated with a fine protein layer that protect them from the stresses coming from their surroundings. One strand of byssus can consist of more than 20 different proteins that are grouped into three groups, including collagen-type proteins that have not been polymerized, matrix proteins and Mussel Foot Protein (MFP) [8]. Byssus helps shellfish to stick firmly to a substrate in water, such as coral or other man-made structures such as ships, docks or ports. They can stick firmly and undisturbed even when physically submerged in water, which is what we know that most adhesives lose their ability underwater.

The study of byssus, especially the protein associated with its attachment, Mussels Foot Protein (MFP), has attracted a lot of interest from scientists and researchers for years. Most researchers applied it in various fields such as biomedicine [9], environment [10] and materials science [11]. One that has been widely reported is the incorporation of MFP as both a matrix and a coating on the surface of the culture substrate of several cell types. The presence of the MFP in the matrix produces several positive impacts on cell growth and cell adhesion. This study will discuss some of the latest developments in MFP applications for tissue culture as well as prospects for future research developments that can be pursued.

2. MUSSEL FOOT PROTEINS (MFPS)

One of the most studied MFPs is the MFP obtained from *Mytilus edulis* or blue clams. This MFP consists of about 85 decapeptides with special residues, the post-translation form of the amino acid tyrosine in the form of a catecholic compound 3,4-dihydroxyphenilalanine or DOPA [12]. The estimated molecular weight of this protein is about 110 kDA with 10% to 15% DOPA residue [7]. This part of DOPA functions as an active site that plays a role in complexation with the substrate, for example ferrous metals which are widely available as minerals in nature. The complexations formed produce unusual mechanical properties with high stiffness but excellent elongation [13]. It was reported that Fe (III) forms remarkably stable complexes with DOPA in the form of bis and tris, depending on environmental conditions, such as surrounding pH.

The byssus is secreted by the foot has unique mechanical characteristics extending in axial orientation along its threads. Tip of the Byssus called plaque (Fig 1a), usually has stronger mechanical properties than the other parts (Fig 1b). This is consistent with the MFP content which shows the highest concentration in plaque [16-25]. At the same time, moving upward towards the distal and proximal parts, the mechanical properties decrease as well as the MFP content [7-19]. These molecular constituent gradients and mechanical properties represent an interesting natural strategy of how shellfish control their biophysical functions.

The strong adhesions generated by this MFP result from the cross-links between the polymer chains of proteins. This process is called hardening, which requires precursors, DOPA and catechol oxidase enzymes [17]. When their foot come out, it secretes protein into the byssus which then forms a template like threads and plaque. The plaque will then stick firmly to the substrate [18]. The reactive DOPA residue will then be oxidized to form quinones which bind to the substrate with water resistance characteristics [19,20]. DOPA is formed from the hydroxylation of the tyrosine residue by enzyme tyrosinase. DOPA can form complexes with several metals such as iron and manganese and semi-metals such as silicon. This is why the presence of DOPA help shellfish stick to the substrate, especially rocks or glass.

3. APPLICATION IN CELL AND TISSUE CULTURE

Culture cell and tissue culture is a modern biotechnological approach that was developed since the late 19th century. The development of technology, especially adequate laboratory equipment, especially in the field of biological and molecular sciences, increasingly supports the development of knowledge about cell culture. The engineering of several types of protein obtained from shellfish species has been widely reported and has the potential in the development of biomaterials or biomedical equipment [21]. Several type of cells cultured with the use of MFP which will be discussed is given below.

3.1 Preosteoblast Cell Culture

Preosteoblast cells are mesenchymal cells that will develop into osteoblasts, where in their development, these osteoblast cells will form bones in the human body. The collection of osteoblast will work together to form the materials needed for bone formation, such as collagen, protein (osteocaltin and osteopontin) and the mineral hydroxyapatite [22]. The preosteoblast cells used for research purposes are generally obtained from mammals such as mice, such as MC3T3-E1 [23]. MC3T3-E1 is a preosteoblast cell isolated from calvarian mice and has been widely used as a model for studying osteoblast differentiation.

Several types of mussel foot protein have been used in MC3T3-E1 cell culture with modifications and engineering performed to increase the effectiveness of cell culture growth. Mussel Foot Protein 1 (MFP-1) isolated from the cuticle layer lining the byssus of the genus Mytilus is reported to be applicable to biomedical implants as a high-performance non-immunogenic coating [24]. Through molecular engineering by constructing MFP-1 with 6 repetitions of decapeptide from Arginylglycylaspartic acid (RGD) peptide, the proliferation of MC3T3-E1 cells can be increased much higher. RGD peptides are peptides that are responsible for the process of attaching cells to an extracellular matrix (ECM), such as fibronectin, collagen, laminin and vitronectin.

Another study used a type of protein in the proximal part of the byssus, namely Proximal Thread Matrix Protein 1 (PTMP-1) obtained from the species *Mytilus galloprovincialis* [25]. The uniqueness of PTMP1 is its residue which has 50% homology with the domain of von Willebrand factor type A (vWF). vWF is a multimeric glycoprotein found in blood plasma, endothelial cells and subendothelial matrix in vessels [26]. vWF is also distributed to other extracellular proteins, including integrins that play an important role in the binding of macromolecules such as collagen as receptor cells. This causes integrins to actively control intracellular processes through the vWF type A domain. The homology of the amino acid residue sequences in PTMP-1 and vWF A was able to mediate the attachment of MC3T3-E1 cells to the collagen matrix and solid substrates commonly used for cell culture (TCPS - Tissue Culture Polystyrene) thereby stimulating the growth of better cell proliferation.

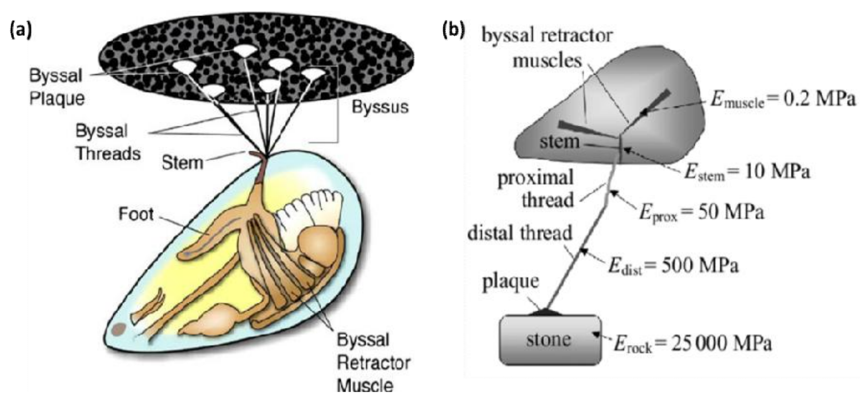

Fig. 1. (a) The shell and anatomical structure of byssus and (b) Mechanical characteristics of byssus [14,15]

3.2 Fibroblast Cell Culture

Fibroblast is a type of cell that synthesizes an extracellular matrix such as collagen which functions as a framework for cell growth and determines cell function and phenotype [27]. Therefore, fibroblasts play an important role in the process of response to wounds and also in their healing. Shortly after the tissue is injured, several molecules with chemotactic activity for fibroblasts begin to form in the area around the wound, including fragments of fibrin, fibronectin and other proteins [28]. The fibroblast cells commonly used in cell culture studies are NIH/3T3 isolated from mice. These cells have long been used as models in cytotoxicity studies [29].

Hwang et al. [30] designed a recombinant protein fp-151 which is a combined hybrid of foot protein 1 (fp-1) and foot protein 5 (fp-5). The growth and adhesion of NIH/3T3 cells is known to be superior to TCPS which is not coated with this protein. However, the growth and cell adhesion performance is still relatively low. Kim [31] perfected the experiment by designing a recombinant protein fp-151 constructed with an RGD motif. This indicates a much better increase in growth performance. Other types of protein obtained from the species *Perna viridis* or green clams were also reported to be potential in increasing the growth of NIH/3T3 cells. *Perna viridis* foot protein 5β (pvfp-5β) coated on the TCPS surface did not show a toxic effect on NIH/3T3 cells and increased cell adhesion up to 6 times higher than controls, namely TCPS that was not coated with anything and coating with cell-tak and PLL (Poly-l-Lysine).

3.3 Keratinocytes Cell Culture

Keratinocytes are the most common cell types found in the epidermis. Nearly 90% of keratinocytes cells are found in this outer layer of humans [32]. Because of its position which is located in the outer part, the function of keratinocytes is as protection from the environment, such as UV, heat and pathogens, both bacteria, fungi and viruses. Research on the cytotoxicity of a substance to human skin generally uses this cell type as a model. In addition, they are also widely used in the development of other applications such as 3D bioprinting, wound healing, implants and soft electronic devices [33-35].

Ahn [36] constructed a recombinant protein fp-151 conjugated with vitronectin (VT), which is a glycoprotein that supports cell attachment and cell spread. The use of this recombinant protein has succeeded in increasing cell proliferation and can show anti-inflammatory activity in keratinocytes cells by preventing the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), interleukin (IL)-1β, interleukin (IL)-6, and Tumor Necrosis Factor (TNF)-α in keratinocytes stimulated with UVB. All of these components are components that will play a role in the inflammatory process, and will increase in expression when cells are exposed to UV-B [37]. However, with the application of this recombinant protein the expression of all these components can be minimized even under UV-B exposure.

4. MFP RESEARCH PROSPECTS AND MUSSEL INSPIRED TECHNOLOGY

Studies on MFP have been carried out with a variety of applications that are not limited to the medical or biomaterials fields. Its use has expanded into multidisciplinary fields. In addition to recombinant proteins which are widely developed for MFP production, biomimetic material technology or commonly called Mussel Inspired Materials, is also mostly done by making MFP as an example in fabricating materials with the same characteristics but from materials that are easily available and environmentally friendly [38]. With the development of biomimetic technology, MFP application dimensions are more affordable. Apart from being a matrix for the synthesis of biomaterials such as collagen, hydroxyapatite and others, this technological approach has been applied in other fields such as water purification from pollutants, bioprinting, soft electronics, biofouling and so on [39].

Indonesia itself as a maritime country and the center of the world's marine megabiodiversity, has a lot of potential for various types of shellfish. It is reported that in Indonesian waters the number of species from the Bivalve Class reaches 1000 but only 18 species have been used either as foodstuffs or others [40,41]. Each species certainly has different characteristics, especially in terms of its properties and protein composition. This can then be studied further to explore new, better candidates with better properties. In addition, recombinant and protein technology biomimetic will also benefit the environment because it will not disturb the stock of the shellfish population in nature, so that its application will be more sustainable.

5. CONCLUSION

MFP and its modification have been reported to give positive results in cell cultures. several examples of MFP application in cell culture such as preosteoblast cells and fibroblast cells and keratinocytes have shown better cell adhesion ability and good cell proliferation ability. The development of MFP applications in the form of recombinant protein and biomimetic materials is a research agenda that can still be developed, especially considering the richness of Indonesian marine biodiversity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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