1. Introduction

Plant cell culture has long been considered as a potential system for large-scale production of secondary metabolites. In recent years, with the advances in plant molecular biology, plant cell culture has also attracted considerable interests as an expression platform for large-scale production of high-value recombinant proteins. Many plant species can now be genetically transformed. Callus cells derived from the transgenic plants can be grown in simple, chemically defined liquid media to establish transgenic cell suspension cultures for recombinant protein production. For certain plant species, such as tobacco, it is also possible to establish transgenic suspension cell cultures by directly transforming wild-type cultured cells. There are several notable benefits of using plant suspension cultures for recombinant protein production. Plant cells, unlike prokaryotic hosts, are capable of performing complex post-translational processing, such as propeptide processing, signal peptide cleavage, protein folding, disulfide bond formation and glycosylation, which are required for active biological functions of the expressed heterologous proteins [1]. Plant cells are also easier and less expensive to cultivate in liquid media than their mammalian or insect cell counterparts. The potential human pathogen contamination problem associated with mammalian cell culture does not exist in plant cell culture since simple, chemically defined media are used [2]. When compared with transgenic plants, cultured plant cells also possess a number of advantages. Cultured plant cells have a much shorter growth cycle than that of transgenic plants grown in the field. Plant cell cultures are grown in a confined environment (i.e. enclosed bioreactor) and hence devoid the GMO release problem. Furthermore, cell suspension cultures consist of dedifferentiated callus cells lacking fully functional plasmodesmata and hence there is minimum cell-to-cell communication. This may reduce systemic post-transcriptional gene silencing (PTGS) which is believed to be transmitted via plasmodesmata and the vascular system [3,4]. On the down side, plant cells generally have a longer doubling time than bacterial or yeast cells. Genetic instability associated with de-differentiated callus cells due to somaclonal variation is another potential drawback in using cultured plant cells for recombinant protein production. Due in part to their more evolved and more tightly controlled gene/protein
regulation machinery, it is more difficult to manipulate protein expression in plant cells, rendering a generally lower protein expression level, normally between 0.1-1 mg L$^{-1}$ of culture [2], although product level as high as 129 mg L$^{-1}$ has also been reported in the case of recombinant human granulocyte-macrophage colony stimulating factor (hGM-CSF) production in transgenic rice cell suspension culture [5].

Plant cell cultures have been used for producing a variety of recombinant proteins. Several research groups have reported expression of antibodies or antibody fragments in plant cell suspension cultures. Some notable examples are the expression of a secretory anti-phytochrome single-chain Fv (scFv) antibody [6], a TMV-specific recombinant full-size antibody [7], a mouse IgG1 recognizing a cell-surface protein of Streptococcus mutants [8], and a mouse scFv [7,9], all using tobacco suspension culture. A number of therapeutic proteins have also been expressed in plant cell cultures, including Hepatitis B surface antigen (HBsAg) [10], human cytokines such as Interleukin IL-2, IL-4 [11], IL-12 [12], and GM-CSF [5,13], ribosome-inactivating protein [14], and human $\alpha$-antitrypsin [15,16]. Readers are also referred to other comprehensive reviews on the subject of recombinant protein expression in plant tissue cultures [2,4,17].

Plant cell culture processes for recombinant protein production resemble conventional recombinant fermentation processes in that they also encompass upstream and downstream processing. However, there are distinctive properties associated with plant cells that call for unique approaches in designing and operating plant cell bioprocesses. The emphasis of this review will be on the upstream processing; specifically, on the engineering considerations associated with the design and operation of bioreactors for recombinant protein production using plant cell suspension cultures. While much of the knowledge derived from the development of plant cell bioreactors for secondary metabolite production are still relevant, issues unique to recombinant protein production will be emphasized in this chapter. New findings since the publications of other recent reviews of plant cell bioreactor [18,19] will be highlighted. Effective bioreactor design and operation assures high productivity which is key to successful bioprocess development. This chapter will begin with an overview of the unique properties of plant cell cultures relevant to bioreactor design. Next, characteristics of recombinant protein expression in plant cell culture are reviewed. This is followed by discussions on a number of key topics relevant to bioreactor engineering, including plant cell bioreactor operating strategies, bioreactor configurations and impeller design, and innovative process sensing, as pertinent to recombinant protein production.

2. Culture characteristics

Plant cell suspension cultures are derived from callus cells. These are unorganized, generally undifferentiated cells [20]. When suspended in liquid media, cells are sloughed off friable calli to form a culture suspension. An effective plant cell suspension culture system for recombinant protein production is expected to possess certain desirable features, including fast growth rate, ease of genetic transformation, high protein expression capacity, low endogenous proteolytic activity, low content of phenolics (which may form complexes with proteins and complicates protein purification) and other phytochemicals (such as oxalic acid) that may interfere with downstream processing, superior post-translational processing capability, and good