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MPG201T Medicinal Plant Biotechnology, M.Pharm Semester 2

Unit 3: Immobilisation Techniques and Secondary Metabolite Production

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3.1 Immobilization techniques of plant cells

Immobilization is the confinement of living plant cells in or on a support so that they stay in place while medium flows around or past them, separating, in engineering terms, the catalyst, the cells, from the product stream. The techniques should be known by family, with the working principle of each. Entrapment, the most used family, encloses cells inside a gel bead or matrix formed around them under mild conditions, classically calcium alginate beads, with agar, agarose, carrageenan and polyacrylamide gels also in the standard repertoire. Adsorption and attachment bind cells to the surface of a support, such as foam, fibres or mesh, by natural adhesion. Encapsulation encloses cells in a membrane bound capsule, a special form of entrapment with a distinct boundary. Covalent binding attaches cells chemically to a support and is the least gentle of the families, so it is least favoured for living plant cells. Whichever family is chosen, the material must satisfy the same conditions: non toxic to cells, porous enough for nutrients, substrates and products to diffuse freely, mechanically stable in a reactor, and cheap and sterilisable at scale. The families are collected in the diagram below.

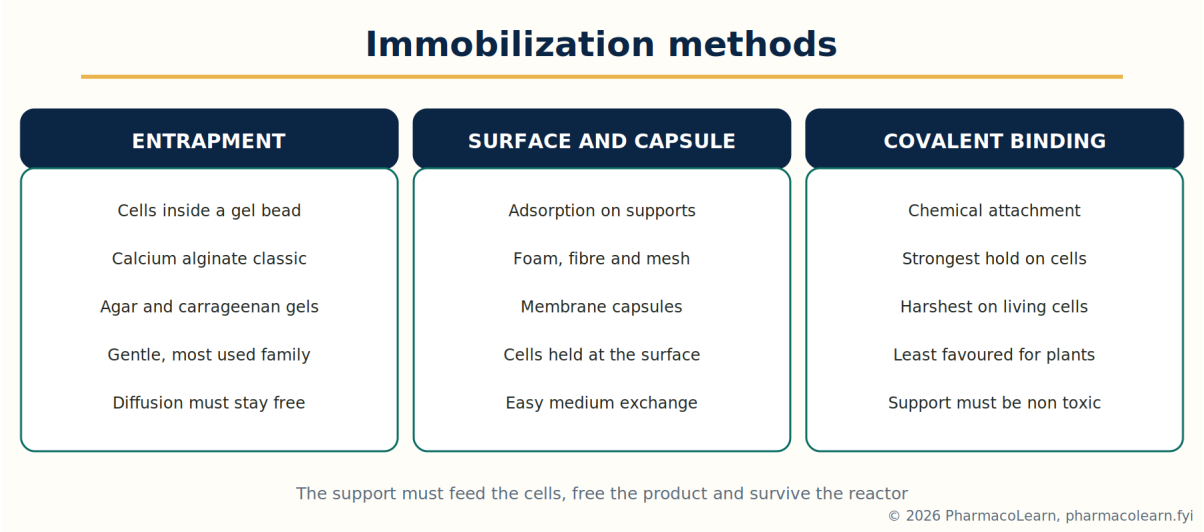

Figure: The immobilization families. Entrapment dominates plant cell work because it is gentle: the gel forms around living cells under conditions they survive.

3.2 Application of immobilization on secondary metabolite production

The application to secondary metabolite production is the reason the technique earns half a unit, and its advantages should be argued, not merely listed. Immobilised cells can be held at high density in a reactor, far denser than free suspensions tolerate, so the producing biomass per volume rises. The cells are protected from the shear forces of stirred systems, a real limit for large, fragile plant cells. Growth and production can be separated: cells are grown first, then held in a production phase under conditions optimised for the metabolite rather than for growth, for as long as they remain productive, and the same cells can be reused over successive production cycles, which free batch culture cannot do. Where the product is released into the medium, it can be drawn off continuously and recovered from a cell free stream, simplifying downstream processing and, by removing the product, relieving feedback restraint on the

pathway. Immobilization also suits biotransformation and precursor conversion work, where the cells act as a reusable enzymatic bed. The disadvantages complete the answer: diffusion through the gel can starve the bead centre of oxygen and nutrients, growth inside beads can break them, the system adds cost and complexity, and products stored inside the cells rather than released gain little from the arrangement. The technique therefore succeeds selectively, for released products, long production runs and shear sensitive lines, and the examination skill is matching those conditions to a case.

3.3 Cloning of plant cells: different methods of cloning

Cloning of plant cells means deriving a population, a cell line, from a single cell or a small uniform group of cells, so that the line is genetically as uniform as culture allows and its properties can be attributed to that origin. The standard methods should be listed with how each secures the single cell origin. In the plating method, a suspension is spread at low density on solid medium so that separated cells or tiny aggregates grow into discrete colonies, each colony being a clone that can be picked and grown on. In the nurse culture and feeder layer methods, single cells that refuse to divide alone are grown over, or separated by a membrane from, a layer of actively growing cells that condition the medium with the factors sparse cells need. Microdroplet and microchamber methods isolate single cells in tiny volumes under observation, so the single cell origin is verified by sight rather than assumed. Cell sorting and selective enrichment complete the practical set, separating cells by size, density or a selectable property before plating. In the wider sense of the unit, cloning also reaches the plant level of Unit 2: a cloned cell line regenerated through embryogenesis gives cloned plants, and micropropagation is cloning practised on shoots. The common purpose across methods is selection: once clones exist separately, each can be assayed for the constituent wanted, and the best becomes the production line, which is the bridge to Section 3.5.

3.4 Applications, advantages and disadvantages of plant cell cloning

The applications of plant cell cloning follow from uniformity plus selection. Cloned lines are the material of metabolite production, because a screened high producing clone gives a reproducible starting point for scale up. They serve biosynthetic and regulation studies, because results from a uniform line can be compared across experiments. They preserve valuable genotypes found in culture, including productive variants thrown up by somaclonal variation, by holding them as defined lines, and, regenerated, they deliver uniform plants for research and production plots. The advantages should be stated plainly: uniformity and reproducibility of the biological material; the power to select at the level of millions of cells for a trait that could never be screened plant by plant in a field; speed, relative to breeding cycles; and containment, since lines are held and tested in the laboratory before any plant is released. The disadvantages are the honest counterweight, and several echo earlier sections: cultured cells drift, cloned lines can lose productivity or accumulate variation over long subculture, the very instability of Section 2.5 working against the uniformity cloning promises; many lines produce less than the parent plant because differentiation dependent genes are off; single cell cloning is technically demanding, with low plating efficiency in many species; and a line selected in a flask still has to prove itself at reactor scale. The balanced verdict an examiner rewards is that cloning is the indispensable selection instrument of the subject, whose products must be stabilised and re verified continuously, not a one time guarantee.

3.5 Secondary metabolism in tissue cultures, with emphasis on production of medicinal agents

Secondary metabolites are the compounds a plant makes beyond the needs of basic growth: the alkaloids, glycosides, terpenoids, phenolics and other families that pharmacognosy exists to study and that form most plant derived medicines. In the intact plant they serve defence and signaling roles, are often made in specialised tissues, stored in specialised structures, and switched on by the regulation systems of Unit 1.

Tissue culture asks whether that chemistry can be run in vitro, and the unit answer has a structure every student should hold. First, the general finding: cultured cells retain the genetic capacity for secondary metabolism, callus and suspension cultures of many medicinal species do produce their characteristic constituents, and organised cultures, hairy roots and shoots of Unit 2, usually reproduce the parent profile more faithfully because differentiation is preserved. Second, the general problem: undifferentiated cultures frequently produce much less than the plant, or stop producing, because the pathway genes need the tissue context, the storage structures, or the signals that culture lacks; growth and production also compete, fast growing cultures often making least product, so production is commonly a two phase affair, a growth phase building biomass and a production phase, with changed medium and conditions, building metabolite. Third, the strategies that raise production, which organise the rest of the unit: select high producing cell lines by the cloning and screening of Sections 3.3 and 3.4; restore differentiation through root or shoot culture; feed precursors and apply elicitors, Section 3.6; immobilise and run extended production, Sections 3.1 and 3.2; and engineer the pathway itself by gene transfer, Unit 4. The standard textbook examples prove the principle qualitatively: shikonin production from cultured Lithospermum cells became the classical demonstration that a plant cell culture could supply a commercial medicinal pigment, and culture production or enhancement has been taught through examples such as ajmalicine from Catharanthus cultures, rosmarinic acid from Coleus cultures and taxol type diterpenes from Taxus cultures, each presented in the texts as a system improved by line selection, medium design and elicitation rather than as a fixed yield. The production flow below states the working sequence.

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Establish callus from the authenticated medicinal plant
        |
Raise a suspension culture and clone single cell lines
        |
Screen the clones and select the high producing line
        |
Growth phase: build biomass under growth conditions
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Production phase: changed medium, precursors, elicitors
        |
Harvest cells or medium; extract and assay the metabolite
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Feed results back: reselect lines, adjust the production phase
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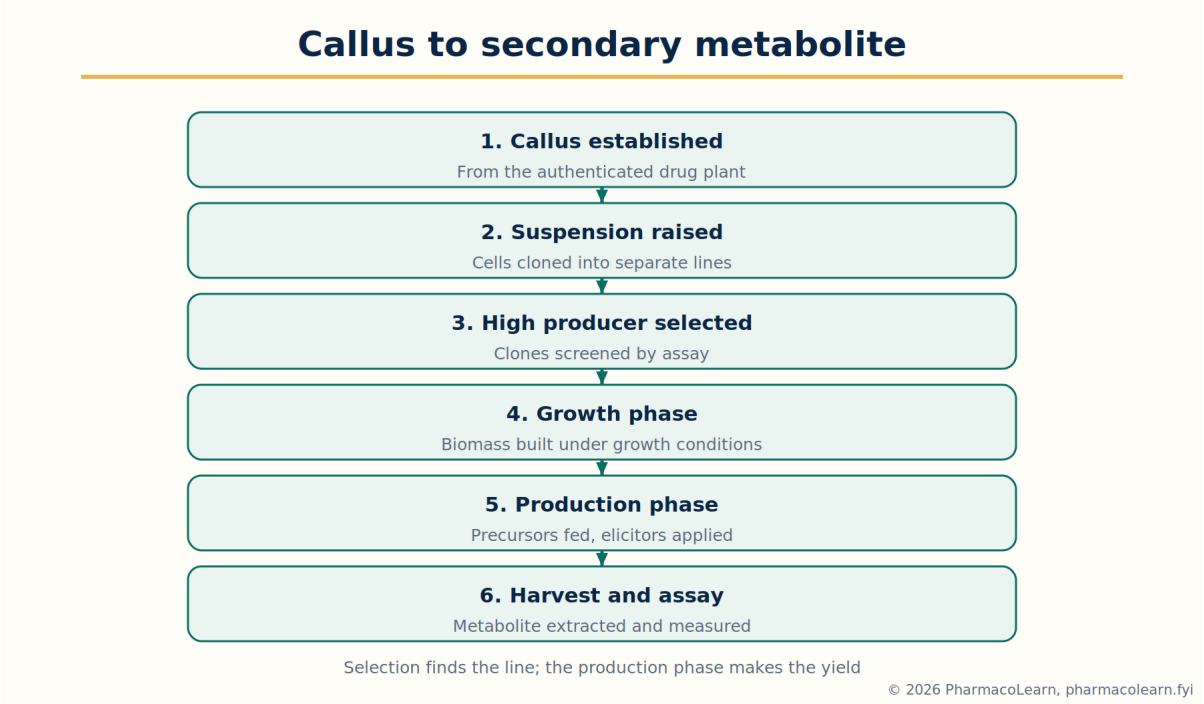

Figure: From callus to metabolite. Selection finds the producing line; the separate production phase, with precursors and elicitors, is where the yield is actually made.

3.6 Precursors and elicitors on production of secondary metabolites

Precursors and elicitors are the two chemical levers of the production phase, and they work by different logic, a distinction the syllabus phrase invites and examiners test. A precursor is a building block of the product pathway, an intermediate or a near intermediate, fed to the culture so the pathway runs faster or further than its own supply allows: amino acid precursors feed alkaloid pathways, acetate and mevalonate units feed terpenoid pathways, and phenylalanine type precursors feed phenylpropanoid products. Precursor feeding succeeds when the fed step is genuinely limiting and the culture can take up and use the compound, and it fails instructively when the true limit lies elsewhere, in enzyme level or storage. An elicitor is a signal, not a building block: a substance or treatment that mimics attack or stress and switches on the defence chemistry of which most secondary metabolites are a part. Elicitors divide into biotic elicitors, of microbial or plant origin, such as fungal cell wall preparations, yeast extract and the signaling molecules of the jasmonate family, and abiotic elicitors, such as ultraviolet light, heavy metal salts and physical stresses in the standard lists. Timing and dose decide success: elicitors are usually applied to a grown culture at the start of the production phase, and excess simply damages cells. Used together, the two levers state the unit in one sentence: precursors supply the bricks, elicitors issue the order to build, and line selection, immobilization and differentiation decide whether the factory receiving them can answer. The comparison is tabulated below.

Feature	Precursor	Elicitor
Nature	Pathway building block or intermediate	Signal that mimics stress or attack
Mechanism	Relieves a supply limit inside the pathway	Switches pathway genes on through signaling
Examples of the class	Amino acids for alkaloids, phenylalanine for phenylpropanoids	Fungal preparations, yeast extract, jasmonates, UV
Applied	During the production phase, fed to the medium	At the start of the production phase, in small dose
Fails when	The real limit is enzyme level, uptake or storage	The dose injures cells, or the line cannot respond