BIOCHEMISTRY

Stem Cells & Neurodegenerative Diseases

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PART 1 — What Are Stem Cells?

Definition

Stem cells are **unspecialized (undifferentiated) primal cells** found in all multicellular organisms. They share the same family (lineage) and are defined by two cardinal properties:

1. **Self-Renewal** — the ability to undergo numerous cycles of cell division while staying undifferentiated, thereby maintaining the stem cell pool.
2. **Differentiation** — the capacity to give rise to a wide range of specialized, fully mature cell types.

KEY CONCEPT

- Stem cells do **NOT** jump straight to a mature cell — they pass through partially-differentiated intermediate cells called **Progenitor Cells** before reaching full maturity.
- Asymmetric division produces ONE renewal daughter + ONE differentiating daughter simultaneously.

PART 2 — Stem Cell Division & The Niche

Asymmetric Division — How Does It Work?

During cell division, **cell membrane proteins** (e.g. receptors) are unequally distributed between the two daughter cells. Proteins that maintain "stemness" segregate to the renewal daughter; proteins that drive differentiation go to the other.

The Stem Cell Niche

The niche is a **specialized microenvironment** that surrounds stem cells and provides everything needed for self-renewal and controlled differentiation.

Niche Component	Description
Cells only	Single type or multiple interacting cells; may be from outside the lineage or from stem cell descendants
Cells + ECM	Extracellular matrix adds structural support and biochemical signals
Secreted / surface factors	Notch, Wnt, FGF, EGF, TGF-β, SCF, chemokines

Functions of the niche:

- **Nutritive** — supplies growth factors and metabolites
- **Feedback control** — regulates pool size (prevents over-expansion or depletion)
- **Lineage coordination** — ensures balanced production of different mature cell types

PART 3 — Potency of Stem Cells

Potency = the ability to differentiate into various cell types (i.e., how many cell types can be produced from a given stem cell).

Potency Type	What Can It Produce?
Totipotent	ALL body cells **+ extra-embryonic tissue** (e.g. placenta). *Most potent.* Example: Zygote.
Pluripotent	ALL body cells from all 3 germ layers — **but NOT** placenta. Example: ESC, iPSC.
Multipotent	Several cell types within a lineage. Example: HSC → all blood cells.
Unipotent	Only **one** cell type. Example: muscle stem cells. *Least potent.*

PART 4 — Types of Stem Cells

A. Embryonic Stem Cells (ESCs)

ESCs are derived from the **inner cell mass (ICM) of the blastocyst** before uterine implantation. They are **pluripotent**.

Development path: Fertilized egg → Zygote → 8-cell embryo → Blastocyst → Inner Cell Mass (*where ESCs reside*)

Pluripotency Transcription Factors in ESCs:

- Oct4
- Nanog
- Wnt-β-catenin signaling
- Other transcription factors

■ Limitation

ETHICAL DILEMMA: Isolating ESCs destroys the embryo → moral/religious concerns.

Also: transplanting ESCs from one individual to another carries **immune rejection risk**.

This drove the search for an alternative — the iPSC.

B. Induced Pluripotent Stem Cells (iPSCs)

iPSCs are generated by **reprogramming fully differentiated adult cells** (e.g. fibroblasts from skin biopsy) back to a pluripotent state. Only a fraction of reprogrammed cells become true iPSCs.

Discovery

Yamanaka (2006/2007) Nobel Prize: Used 4 transcription factors to reprogram fibroblasts: OCT3/4 • SOX2 • c-Myc • KLF4

Thomson group used a different set of 4 TFs and achieved the same result simultaneously.

Yamanaka compared iPSCs vs ESCs across all these parameters — they were indistinguishable:

Morphology	Gene expression	Surface antigens	Telomerase activity
Proliferation rate	In vitro differentiation	Teratoma formation*	Epigenetic status

* A **teratoma** is a tumor containing cells from all 3 germ layers — used as a gold-standard test of pluripotency.

iPSC Advantage	Explanation
Ethical ✓	No embryo is destroyed
Autologous ✓	From the same patient → no immune rejection
Patient-specific ✓	Mirrors the patient's own genetic makeup
Safer ✓	No immunological rejection issues

C. Adult Stem Cells (ASCs)

Found throughout the body after birth. They are generally **multipotent or unipotent**. Primary function: **replenish dying cells and regenerate damaged tissue**.

Type	Key Features & Derivatives
1. Bone marrow — HSC	Hematopoietic Stem Cells → ALL blood cell types (lymphoid + myeloid lineages)
1. Bone marrow — MSC	Mesenchymal Stem Cells → osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells
2. Neural (NSC)	Neurospheres in subventricular zone (SVZ) + dentate gyrus of hippocampus → oligodendrocytes, neurons, astrocytes
3. Adipose (ASC)	From fat tissue after liposuction → fibroblasts, pericytes, endothelial cells
4. Umbilical cord	Cord blood: HSC | Cord tissue: MSC
5. Olfactory	In olfactory mucosa → regenerate sensory cells damaged by chemicals/odorants
6. Cornea/trabecular	Located at cornea-iris junction → regulate intraocular pressure (near Schlemm's canal)

PART 5 — Uses & Limitations of Stem Cells

Use	Detail
Study differentiation signals	Understand how cell fate decisions are made in normal/disease contexts
Drug testing	Differentiate into target cells (e.g. hepatocytes) to test drug efficacy and safety in specific genotypes
Genetic therapy	Correct mutations before re-implanting patient-derived cells
Cell-based therapies	Replace lost or damaged cells in patients
Cancer treatment	Activate chemotherapeutic agents locally at tumor sites

Limitations of Stem Cell Therapy

PROS ✔
• Can treat many different diseases
• Avoids need for donor transplant (if autologous)
• iPSC = no immune rejection
• Can model disease in vitro

CONS ✗
• Carcinogenicity (risk of tumor if undifferentiated cells remain)
• Immune rejection (if allogeneic / not autologous)
• Risk of infection during procedures
• Lack of reliable stem cell markers for isolation
• In vitro culture conditions poorly defined
• In vivo niche regulation not fully understood
• Limited potency of adult stem cells vs. pluripotent

PART 6 — Neurodegenerative Diseases Overview

Neurodegenerative diseases involve the loss of neurons and/or glial cells in the brain and spinal cord. They may be:

Category	Examples	Nature of Damage
Acute	Ischemic stroke, Spinal cord injury	Focal, rapid — often single event
Chronic	Parkinson's (PD), Alzheimer's (AD), ALS	Progressive, multi-system degeneration

Key Considerations Before Using Stem Cells for Any Neurodegenerative Disease:

Consideration	Key Points
Clinical competitiveness	Must outperform existing treatments; side effects must be acceptable
Degree of disability	Higher disability → smaller improvement acceptable; mild disease → higher bar
Cell type needed	PD: dopaminergic neurons | ALS: motor neurons | AD/Stroke: multiple cell types
Animal model evidence	Substantial functional improvement must be shown first
Biological mechanism	Understand HOW repair occurs (cell replacement? niche activation? circuitry reconstruction?)
Clinical trial design	Double-blind placebo; realistic informed consent; inclusion/exclusion criteria
Immunosuppression	Brain is immunologically privileged but barrier can be compromised in disease
Administration safety	Route: systemic IV vs. lumbar puncture vs. stereotactic injection

Parkinson's Disease (PD)

Pathology	Loss of nigrostriatal **dopaminergic (DA)** neurons
Symptoms	Rigidity, hypokinesia, tremor, postural instability
Current Tx	L-DOPA, DA agonists, enzyme inhibitors, deep brain stimulation
Gap	No treatment for dementia component
iPSC role	Modeling genetically complex PD in vitro

Stem Cell Therapy Pros & Cons for PD:

PROS ✔
• Transplanted DA neurons reinnervate the striatum and restore DA release
• Some patients showed clear symptomatic relief
• Grafts remain functional for 11–16 years post-transplantation
• Multiple DA neuron sources available (ESC, iPSC, NSC, SVZ, bone marrow)

CONS ✗
• A small fraction of graft neurons develop Lewy bodies (disease pathology may spread)
• Limited availability of human embryonic mesencephalic tissue
• Highly variable functional outcomes between patients
• Poor standardization of transplanted cell material
• PD is multisystem — non-dopaminergic areas won't improve
• Risk of tumor formation is NOT acceptable for a manageable disease
• Need to inject at ALL sites of injury

Clinical Trial Note

ISCO clinical trial used parthenogenetic cells from unfertilized oocytes.

Drawback: Cells were PAX6-positive — but authentic midbrain DA neurons come from PAX6-**negative** precursors. This mismatch is a concern for long-term function.

B. Alzheimer's Disease (AD)

Pathology	Neuronal/synaptic loss, neurofibrillary tangles, β-amyloid deposits
Regions affected	Basal forebrain cholinergic system, amygdala, hippocampus, cortex
Key feature	Tissue-level damage (NOT single cell type) → therapy is more complex

Stem Cell Approaches for AD:

Approach	Mechanism
1. Cholinergic replacement	Acetylcholinesterase inhibitors enhance cholinergic function → temporary improvement only
2. Hippocampal neurogenesis	Promote maturation of immature hippocampal neurons (already found in AD)
3. NGF-releasing stem cells	Stem cells modified to secrete Nerve Growth Factor → stimulate neuron repair
4. Anti-amyloid strategies	Anti-β-amyloid antibodies or β-amyloid-degrading protease neprilysin

Hurdles in AD

- Must pre-differentiate stem cells into MANY different neuroblast types for multiple brain areas.
- Cholinergic replacement requires intact TARGET (host) neurons — which are also damaged in AD.
- Stem cell replacement strategies are **very far** from clinical application in AD.
- Stemedica trial: healthy donor stem cells tested in mild-to-moderate AD patients (ongoing).

C. Ischemic Stroke

Cause	Occlusion of a cerebral artery → focal cell death
Cells lost	Multiple neuron types + oligodendrocytes + astrocytes + endothelial cells (depends on artery)
Recovery	Neuronal plasticity contributes to partial recovery; most patients have persistent deficits

Stem Cell Therapies Tested for Stroke:

Approach	Result/Status
ES-derived NSC + MSC grafts	Migrated toward lesion in rats, improved forelimb performance
IV injection of human NSCs	Improved outcome in hemorrhagic stroke in rats via anti-inflammatory effects
IV autologous MSCs in humans	No substantial clinical improvement detected (MCA lesion patients)
Intraarterial bone marrow cells	Several ongoing trials; none yet established as definitive therapy
Conditionally immortalized NSCs	From human fetal cortex — in clinical trial phase

Important Finding

- 80% of neuroblasts/neurons die within the first 2 weeks after formation at a stroke site in rats.
- Non-neuronal MSCs provide trophic support to enhance endogenous repair and neurogenesis.

D. Spinal Cord Injury (SCI)

Pathological changes	1. Interruption of ascending/descending pathways 2. Loss of neurons + glial cells 3. Inflammation 4. Scar formation 5. Demyelination
Symptoms	Loss of movement, sensation, and autonomic control below injury level
Current treatments	Largely ineffective
Similarity to AD	Tissue-level damage → complex multi-cell therapy needed

Stem Cell Approaches for SCI:

Approach	Mechanism/Status
Formation of neurons, oligodendrocytes & astrocytes	Replace multiple lost cell types
Axon & synapse formation	Reconstruct neural circuitry
Remyelination (OPC)	High-purity oligodendrocyte progenitor cells from human ES cells can differentiate into oligodendrocytes (clinical trial)
Umbilical/bone marrow HSC + MSC	Applied in patients with claims of partial recovery — not yet definitive

Problems in SCI trials:

- Implanted cells were often poorly characterized
- Preclinical evidence of efficacy was insufficient for many approaches
- Improvements may reflect physiotherapy, NOT stem cells (open-label designs)
- Underlying mechanisms of observed improvements were unclear

Before moving to clinic, must determine:

- How to control proliferation of transplanted cells and their progeny
- How to direct differentiation to specific lost neuron types
- How to guide resulting neurons to form appropriate synaptic contacts

PART 8 — Bench-to-Bedside Pathway

Stage	What Happens
In vitro characterization	Cell behavior, potency, gene expression, safety — Year 0–1
Small animal models	Rodent models — tumorigenesis, cell incorporation, animal survival — Year 1–2
Large animal validation	Closer to human physiology — Year 2–4
Human clinical trials (Phase I-III)	FDA + IRB approval, informed consent, inclusion/exclusion, 3 safety/efficacy phases — Year 5–15+
Post-market follow-up	Data Safety Monitoring Board review — Year 15–20+

■ CRITICAL SUMMARY

- **No stem cell-based therapy has yet been proven beneficial for any neurodegenerative disease.**
- Unproven treatments are offered at clinics worldwide — always check for FDA/regulatory approval.
- Ethical, regulatory, societal, and economic issues must all be addressed.
- As a clinician: ALWAYS verify FDA approval and guide your patient to evidence-based choices.

PART 9 — High-Yield MCQ Practice (20 Questions)

Q1. Which of the following BEST describes self-renewal in stem cells?

A. Differentiation into multiple specialized cell types
B. Ability to undergo repeated cell divisions while staying undifferentiated ✓
C. Forming teratomas when transplanted in vivo
D. Responding to paracrine niche signals

Answer: B — Self-renewal = staying undifferentiated through multiple division cycles.

Q2. Asymmetric stem cell division is driven by:

A. Symmetric segregation of telomeres
B. Differential segregation of cell membrane proteins between daughter cells ✓
C. Random epigenetic modification of daughter nuclei
D. Equal distribution of organelles

Answer: B — Stemness-maintaining vs differentiation-driving membrane proteins segregate asymmetrically.

Q3. A stem cell that can give rise to all body cells AND placenta is called:

A. Pluripotent
B. Multipotent
C. Unipotent
D. Totipotent ✓

Answer: D — Totipotent = whole body + extraembryonic tissues. Pluripotent excludes placenta.

Q4. Embryonic stem cells are derived from:

A. The outer trophoblast layer of the blastocyst

B. The inner cell mass of the blastocyst ✓
C. The 8-cell embryo stageD. The fetal liver at week 8

Answer: B — ESCs come from the inner cell mass (ICM) of the blastocyst, before uterine implantation.

Q5. Yamanaka's 4 transcription factors used to generate iPSCs from fibroblasts are:

A. Nanog, Sox2, c-Myc, Wnt
B. OCT3/4, SOX2, c-Myc, KLF4 ✓
C. Oct4, Nanog, TGF-β, KLF4
D. PAX6, OCT4, SOX2, EGF

Answer: B — Yamanaka (2006) Nobel Prize. The 4 Yamanaka factors are: OCT3/4, SOX2, c-Myc, KLF4.

Q6. Which of the following is an advantage of iPSCs over ESCs?

A. Higher pluripotency potential
B. No ethical concerns and no immune rejection risk ✓
C. Easier to isolate from the blastocyst
D. More stable genome

Answer: B — iPSCs are autologous (from the patient), so no rejection, and no embryo is destroyed.

Q7. A teratoma is used to test pluripotency because it:

A. Grows rapidly in culture
B. Contains cells from only ectodermal lineage
C. Contains cells derived from all three germ layers ✓
D. Shows self-renewal capacity

Answer: C — True pluripotent cells form teratomas containing tissues from all 3 germ layers.

Q8. Neural stem cells (NSCs) responsible for adult neurogenesis are found in:

A. The cerebellum and basal ganglia
B. Subventricular zone (SVZ) and dentate gyrus of hippocampus ✓
C. The corpus callosum and thalamus
D. The substantia nigra exclusively

Answer: B — NSC neurospheres are found in the SVZ (lining lateral ventricles) and dentate gyrus.

Q9. Which adult stem cells can be harvested after liposuction?

A. Neural stem cells
B. Hematopoietic stem cells
C. Adipose-derived stem cells (ASCs) ✓
D. Olfactory stem cells

Answer: C — ASCs are mesenchymal stem cells found in adipose tissue, accessible via liposuction.

Q10. The umbilical cord contains which two types of stem cells?

A. ESC in blood + NSC in tissue
B. HSC in cord blood + MSC in cord tissue ✓
C. iPSC in blood + ASC in tissue
D. Totipotent cells in blood + multipotent in tissue

Answer: B — Cord blood = hematopoietic stem cells; cord tissue = mesenchymal stem cells.

Q11. The main pathology of Parkinson's disease that stem cells aim to correct is:

A. β-amyloid deposition in the cortex
B. Loss of motor neurons in the spinal cord
C. Degeneration of nigrostriatal dopaminergic neurons ✓
D. Demyelination of corticospinal tracts

Answer: C — PD = nigrostriatal DA neuron loss. Current therapy: L-DOPA, DA agonists, deep brain stimulation.

Q12. A major hurdle preventing stem cell therapy for PD from reaching the clinic is:

A. DA neurons cannot survive for more than 6 months post-transplant
B. PD is a multisystem disorder — non-DA areas won't benefit from DA grafts ✓
C. iPSCs cannot be differentiated into DA neurons
D. The striatum cannot be accessed surgically

Answer: B — PD affects multiple systems. Only dopaminergic function might improve with DA neuron grafts.

Q13. Alzheimer's disease is particularly difficult for stem cell therapy because:

A. The disease only affects a single cell type
B. It involves tissue-level damage across multiple brain regions and cell types ✓
C. There are no animal models of AD
D. Stem cells cannot cross the blood-brain barrier

Answer: B — AD affects multiple cell types across many areas (hippocampus, cortex, amygdala, etc.).

Q14. In ischemic stroke, which cell types are typically lost?

A. Only neurons
B. Only oligodendrocytes
C. Multiple types: neurons, oligodendrocytes, astrocytes, and endothelial cells ✓
D. Only endothelial cells

Answer: C — The artery territory determines which cell types die — typically multiple cell types are involved.

Q15. High-purity oligodendrocyte progenitor cells (OPCs) from human ES cells can:

A. Only form astrocytes in vivo
B. Differentiate into oligodendrocytes and assist in remyelination ✓
C. Replace lost dopaminergic neurons in PD
D. Produce NGF to slow Alzheimer's progression

Answer: B — OPC-based remyelination is one stem cell approach for spinal cord injury.

Q16. Which of the following stem cell types is MOST potent and genetically engineered with no immune risk?

A. Embryonic stem cells (ESC)
B. Adult neural stem cells
C. Induced pluripotent stem cells (iPSC) ✓
D. Adipose stem cells

Answer: C — iPSCs are pluripotent, patient-specific, genetically correctable, and carry no immune rejection risk.

Q17. The ideal design for a clinical trial testing stem cell therapy is:

A. Open-label observational study
B. Single-arm dose-escalation trial
C. Double-blind placebo-controlled study ✓

D. Retrospective cohort analysis

Answer: C — Double-blind placebo prevents bias and ensures any improvement is due to the therapy itself.

Q18. Stem cells need a niche PRIMARILY because:

A. They require a sterile environment away from immune cells

B. They need specialized support for viability, self-renewal, and lineage coordination ✓

C. They cannot survive in oxygenated conditions

D. They divide too fast without physical restraint

Answer: B — Niche = nutritive support + feedback control of pool size + lineage coordination.

Q19. A drawback of the ISCO PD clinical trial using parthenogenetic cells was that:

A. Cells were PAX6-negative, unlike authentic DA neurons

B. Cells were PAX6-positive, while authentic midbrain DA neurons derive from PAX6-negative precursors ✓

C. Cells failed to survive more than 1 week post-transplant

D. They caused severe immune rejection in all patients

Answer: B — PAX6+ cells suggest dorsal neural fate; DA neurons should come from PAX6-negative ventral midbrain.

Q20. Which statement about translating stem cell therapy from bench to bedside is CORRECT?

A. It typically takes only 2-3 years from in vitro work to clinical use

B. Animal models fully predict toxicity in humans

C. The clinical trials stage alone can take 10-20 years ✓

D. FDA approval is optional if animal data is strong

Answer: C — Bench-to-bedside: in vitro → small animals → large animals → 3-phase clinical trials (10-20 yrs).