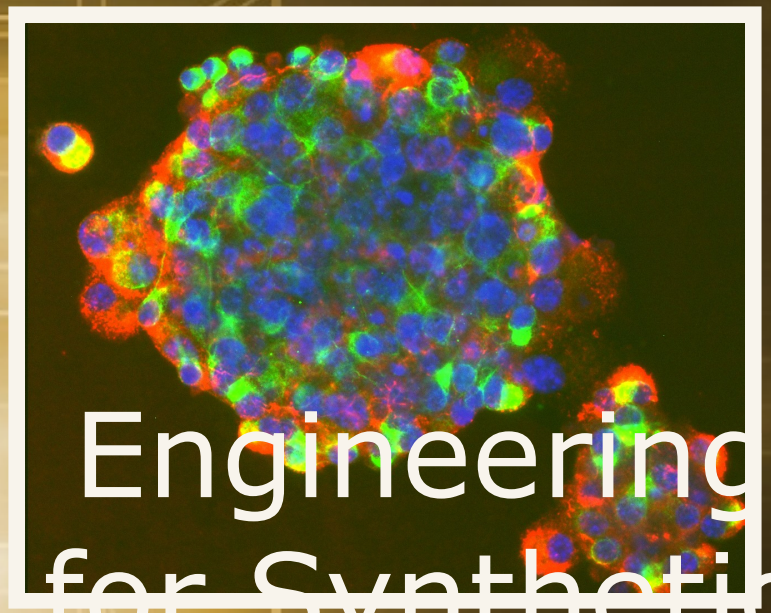
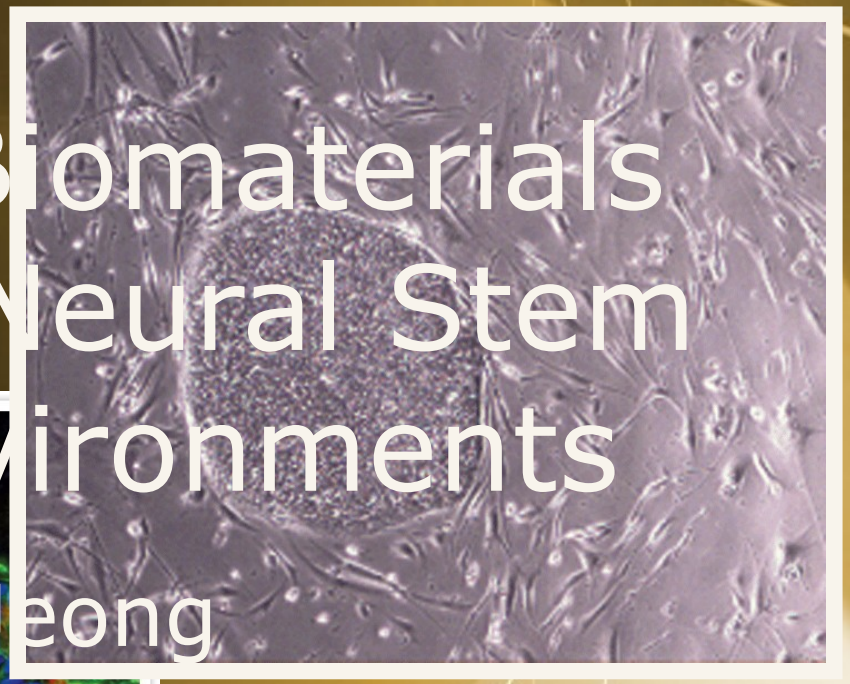
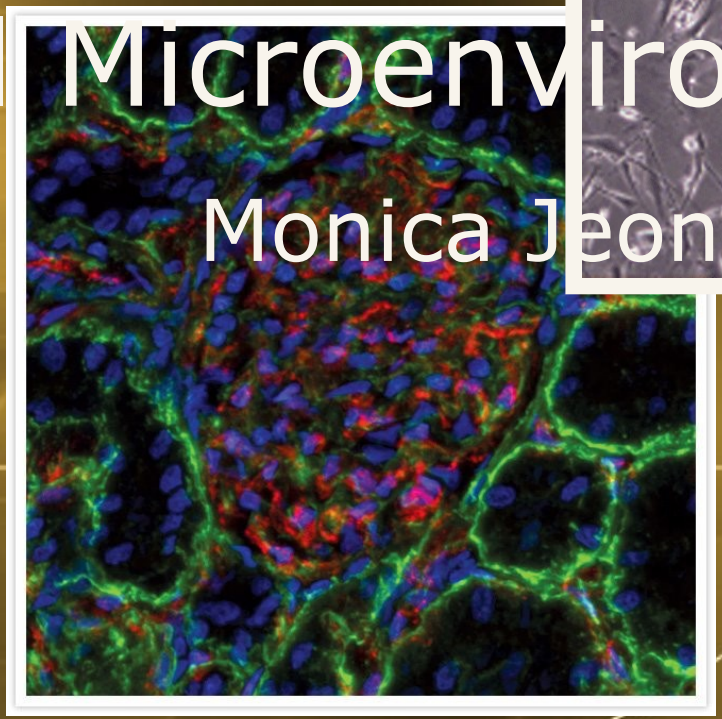


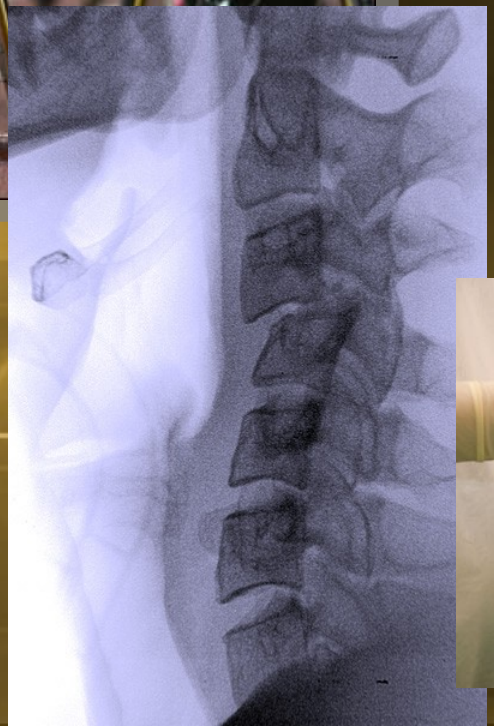
Engineering Biomaterials for Synthetic Neural Stem Cell Microenvironments





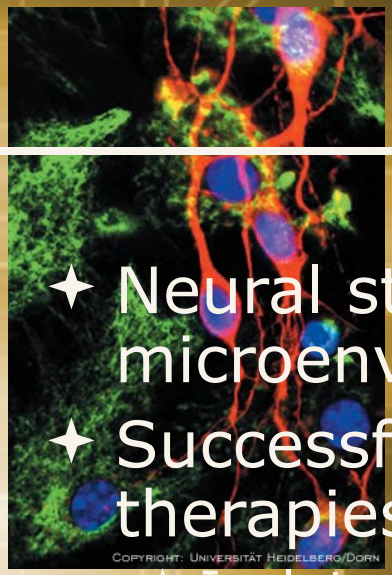
Monica Jeong

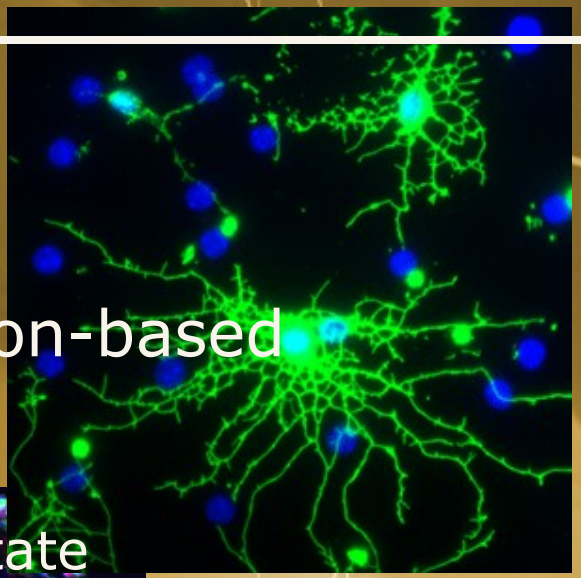
Overview of SCI

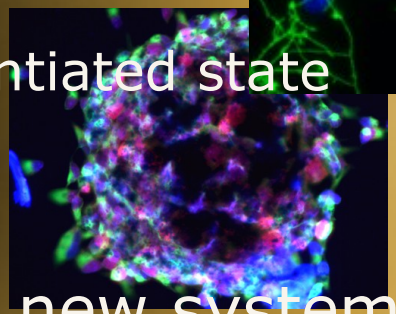


- Spinal Cord Injury
- Severity ranges from complete paraplegia to incomplete myelopathy or paraparesis

Introduction



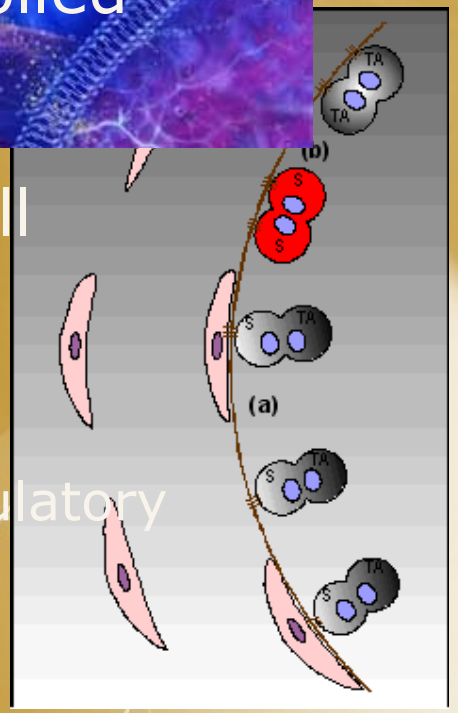




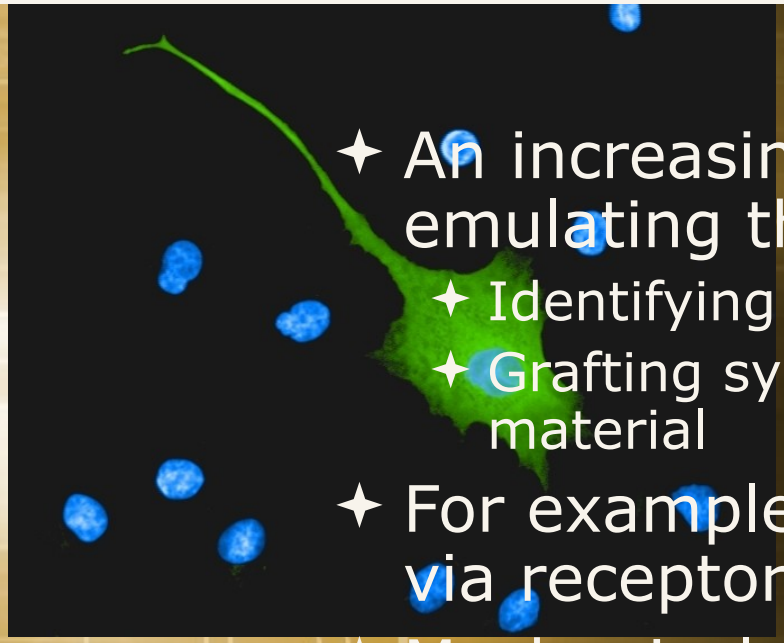
- Neural stem cells (NSC) and the microenvironment or niche
- Successful novel cell transplantation-based therapies:
  - Isolate stem cells
  - Expand them in an undifferentiated state
  - Induce their differentiation
  - Engraft them in vivo
- There is a need to develop new systems or synthetic microenvironments that encourage successful incorporation, survival, and integration of NSCs into diseased and injured regions of the CNS.

Synthetic Microenvironments

- Two major components:
  - Soluble phase
  - Solid phase
- Exploiting molecules to construct controlled stem cell microenvironments has been difficult.
- Matrices or substrates used for stem cell culture or implantation should be:
  - biochemically well-defined
  - purified
  - be bioactive via the presentation of key regulatory signals, nontoxic, nonimmunogenic
  - not pose risks of pathogen transfer.



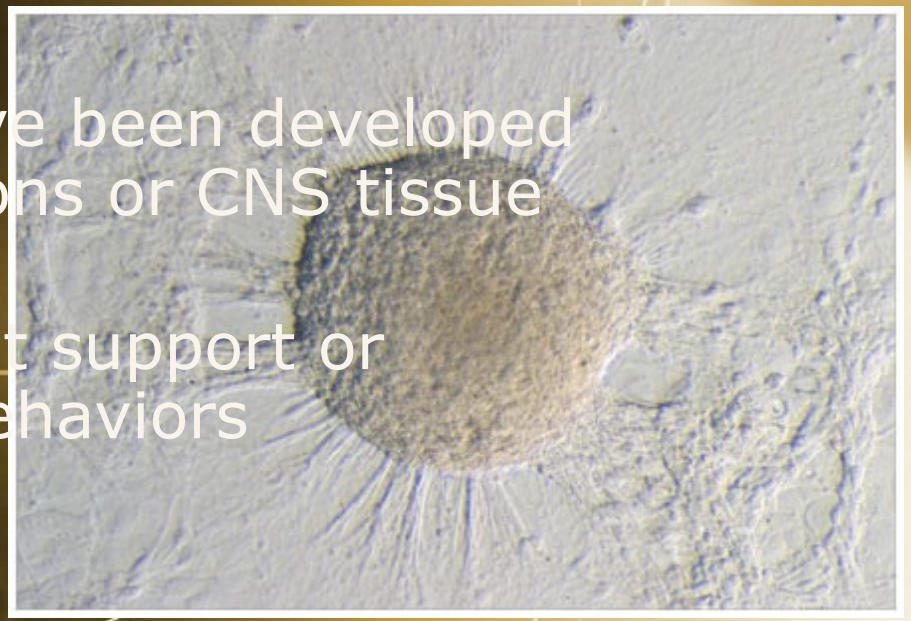
Emulating the ECM



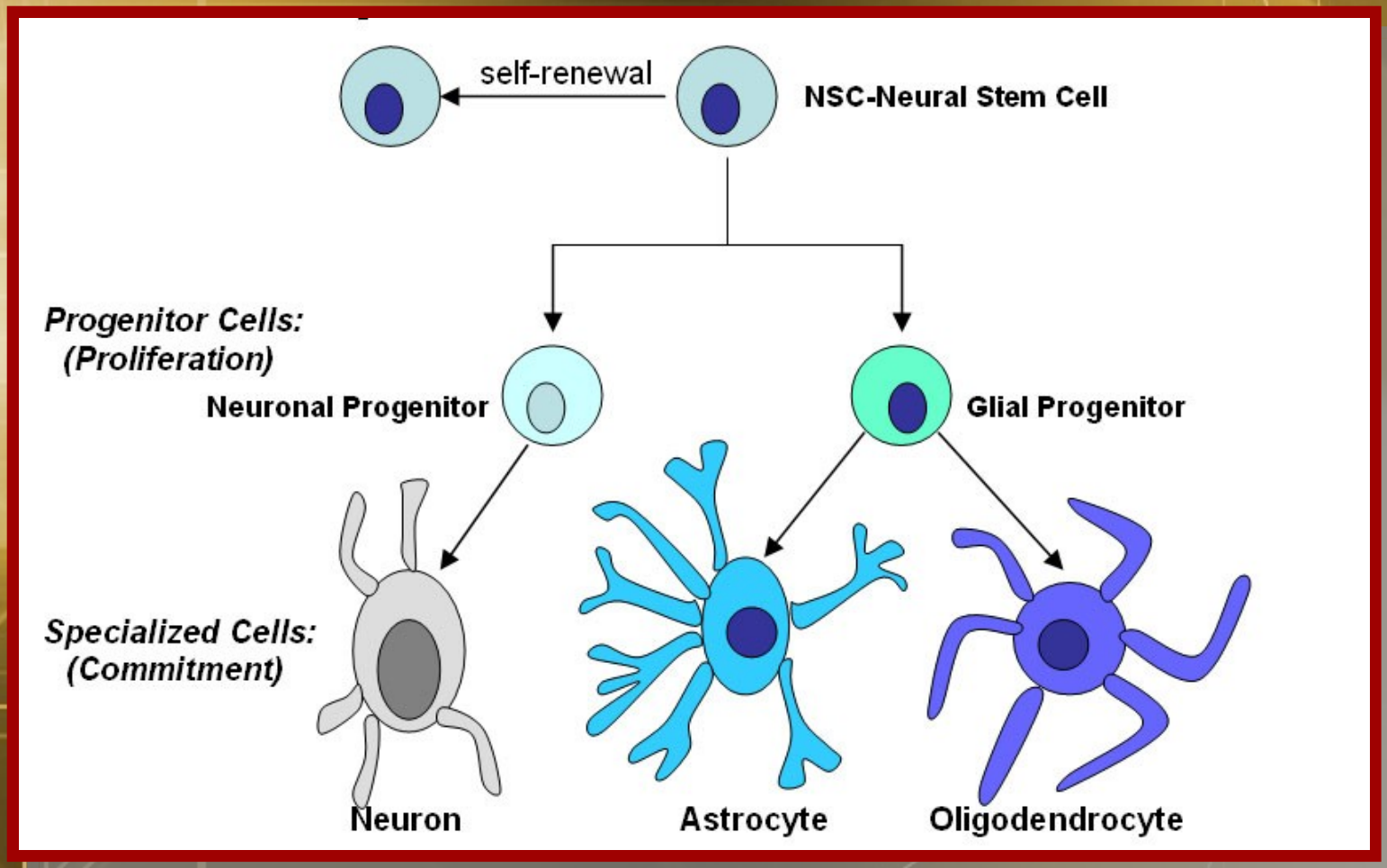
- An increasingly employed approach for emulating the ECM involves…
  - Identifying bioactive motifs
  - Grafting synthetic analogues of these signals onto a material
- For example, cells engage with ECM ligands via receptors such as integrins
- Mechanical properties of the culture system should also be considered.
- The stem cell microenvironment plays a major role in controlling first the expansion and then the differentiation of stem cells for clinical applications.

In Vitro Studies

- "neural stem cell"--a population of cells with the capacity for extended self-renewal or proliferation in an immature state, as well as multipotent differentiation into neurons and glial cells.
- All of these cell populations can be grown either as neurospheres or as an adherent monolayer.
- 2D surfaces or 3D gels have been developed for culturing NSC populations or CNS tissue explants.
- Engineering substrates that support or regulate specific cellular behaviors

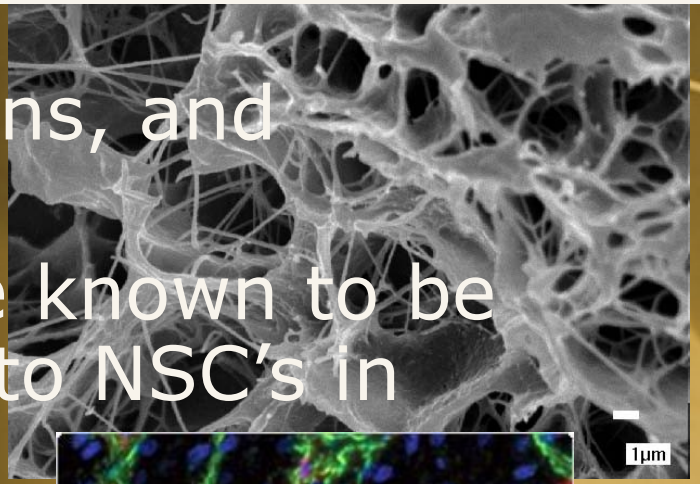


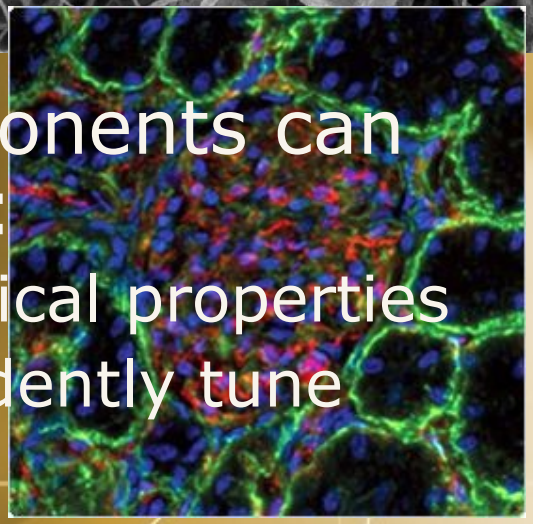
NSC Differentiation

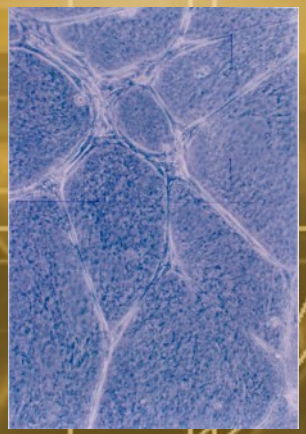


Natural Surfaces and Gels

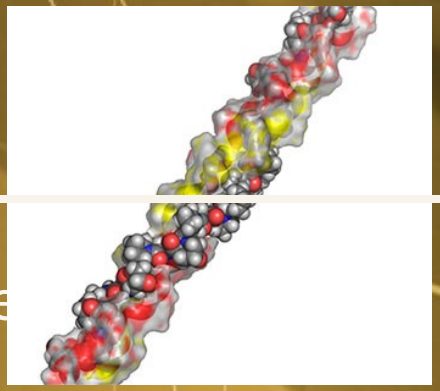
- Collagen, other ECM proteins, and calcium alginate
- Several ECM molecules are known to be present in close proximity to NSC's in vivo.
  - However, natural components can face several challenges:
    - Difficult to tune mechanical properties
    - Not possible to independently tune signals
    - Purity
    - Availability of large-scale sources



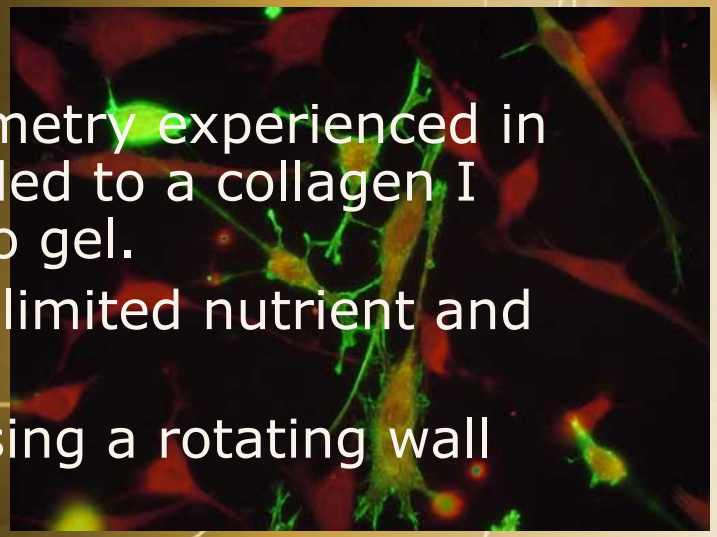




Collagen

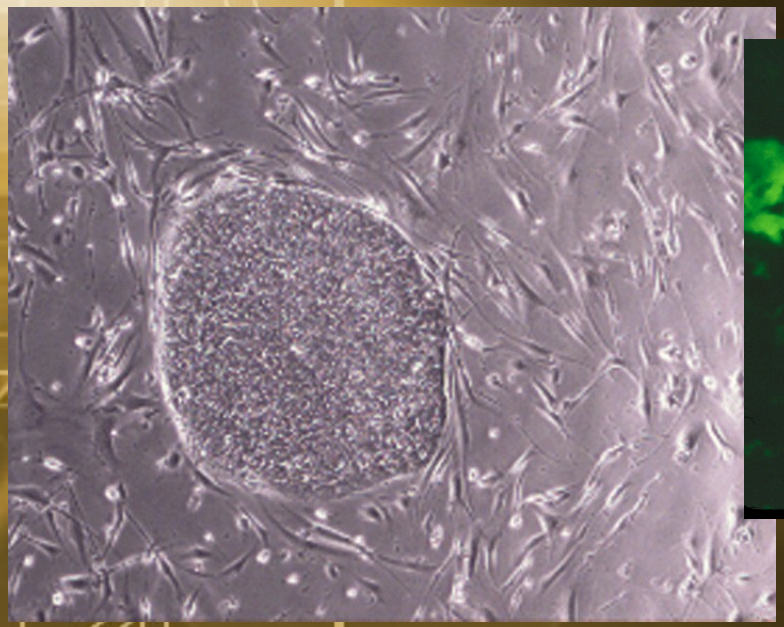


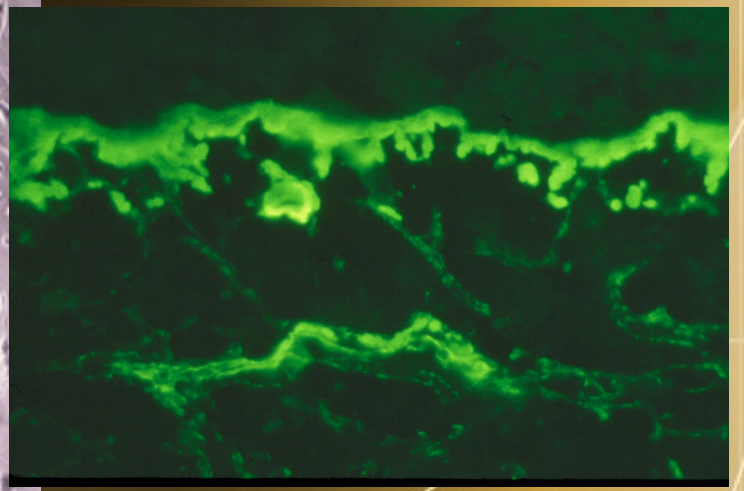
- Triple helix protein that accounts for approximately 30% of all protein found in vertebrate animals
- Present in skin, connective tissue, and many other regions throughout the body.
- Numerous efforts have used 3D type I collagen, which can form gels, to culture rat embryonic cortical NSCs.
  - O'Conner et al.
  - Ma et al.
- 3D gels may better mimic the geometry experienced in vivo. Therefore, cell have been added to a collagen I solution, which was then allowed to gel.
  - High levels of dead cells due to limited nutrient and oxygen transport
  - But cell viability improved by using a rotating wall vessel (RWV) reactor.



Other ECM Molecules

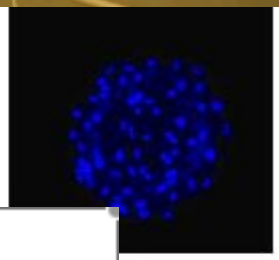
- Matrigel: laminin, collagen IV, and heparan sulfate
- E-C-L attachment matrix: entactin, collagen IV, and laminin

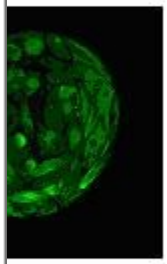


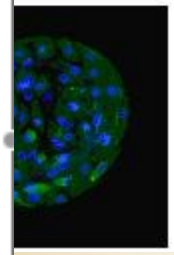


Other ECM Molecules

- Whittemore et al. (
  combinations of EC
  rat SVZ NPC propa







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Mitogen and Substrate Differentially Affect the Lineage Restriction of Adult Rat Subventricular Zone Neural Precursor Cell Populations

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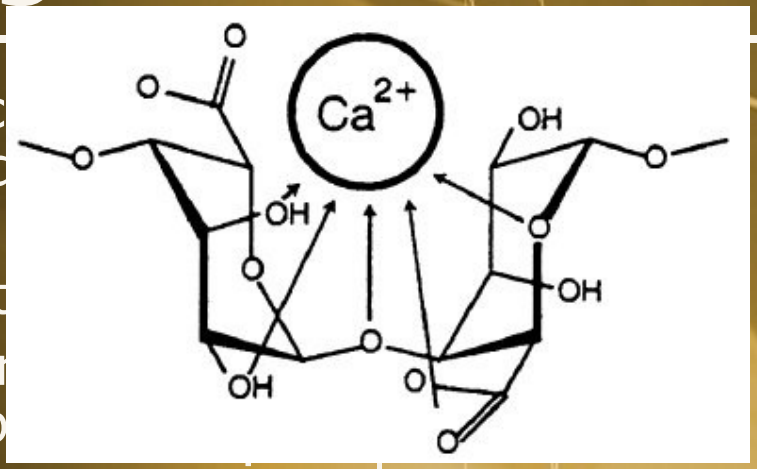
The effects of specific mitogens and substrates on the proliferative capacity and the differentiated phenotypic plasticity of neural precursor cell populations isolated from the adult rat subventricular zone (SVZ) were examined. SVZ cells were grown on uncoated tissue culture plastic, extracellular matrix, or poly-D-ornithine with either laminin or fibronectin. SVZ neural precursor cells could not be generated with platelet-derived growth factor (PDGF), granulocyte macrophage colony stimulating factor, stem cell fac-

INTRODUCTION

The majority of neurogenesis in the mammalian CNS ends early in postnatal life [1]. However, neural precursor cells in both the hippocampal granule cell layer [2–4] and the subventricular zone (SVZ) of the lateral ventricle [5–7] continue to proliferate into adulthood. The fate of these dividing precursor cells has been well characterized. In the SVZ, these cells differentiate into neurons, astrocytes, and oligodendrocytes [6]. The neuronal precursors migrate into the

Calcium Alginate



- Alginates are polyanionic polysacc
  isolated from brown sea algae and
  and guluronic acids
- Gel in the presence of bivalent cat
- Li et al. encapsulated mouse embr
  NPCs in calcium alginate microcap
  proliferated and maintained nestin expression along with the ability to differentiate into neurons and glial cells.
- NSCs were seeded in calcium alginate gels for 7 days in serum-containing medium and then transplanted in rat brain slice cultures. The resulting brain slices exhibited GFP-expressing glial cells and neurosn with axons aligning along the capillary features of the gel.

Semisynthetic Surfaces and Gels

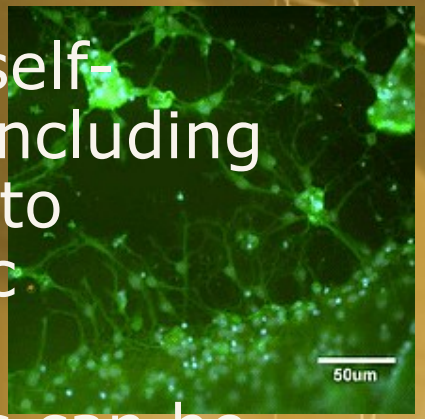
- A blend of synthetic and natural components
- Natural component is typically an ECM protein that is adsorbed to the synthetic component and presents signals to modulate cell attachment, growth, and differentiation.
- Addition of a synthetic component enables control over the architecture and mechanics of the materials.

Fully Synthetic Surfaces and Gels

- Natural components pose difficulties.
- Materials composed of primarily synthetic components offer advantages including low immunogenicity, reproducible and scaleable synthesis, and the ability to tune mechanical and biochemical properties.
- However, biofunctionalizing synthetic material can be challenging.

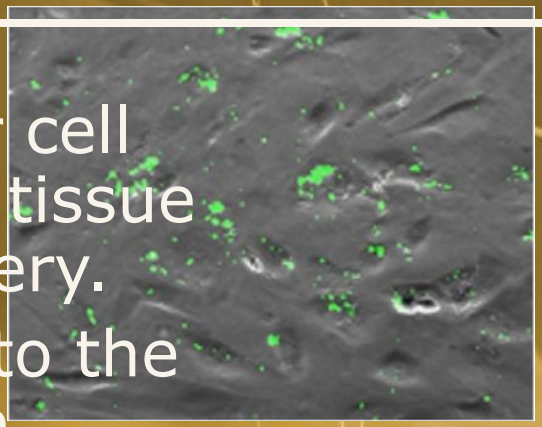
Self-Assembling Peptides

- Some polypeptide sequences can self-assemble into various structures, including beta sheets via hydrogen bonding to cylindrical micelles via hydrophobic interactions.

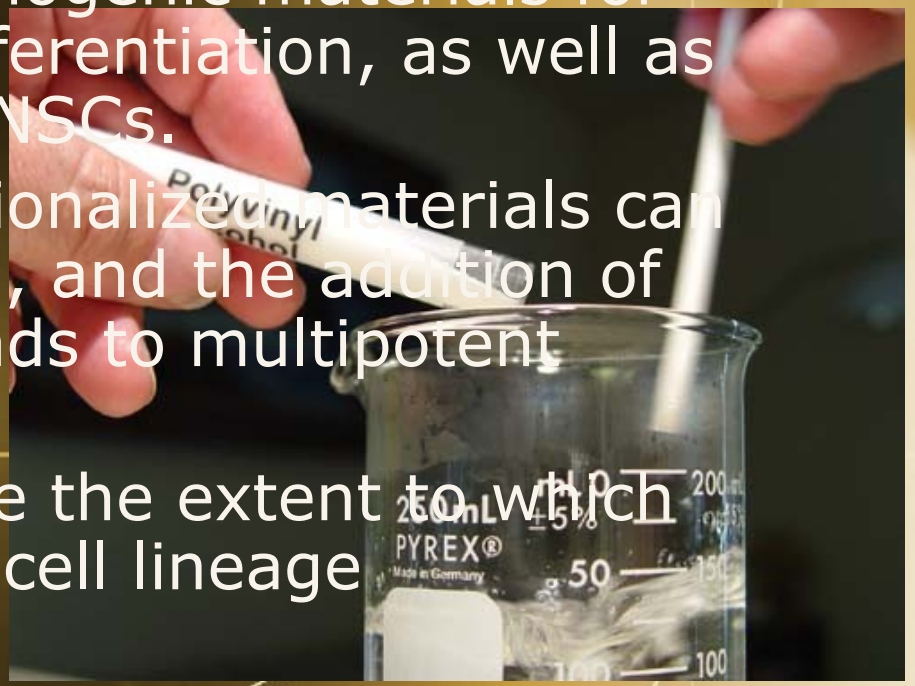


- Self-assembling peptide sequences can be synthesized as fusions to motifs found in ECM proteins, including RGD and IKVAV from fibronectin and laminin to create self-assembled structures that can engage cellular adhesion receptors.
  - Ex. Triblock protein containing an RGDS motif

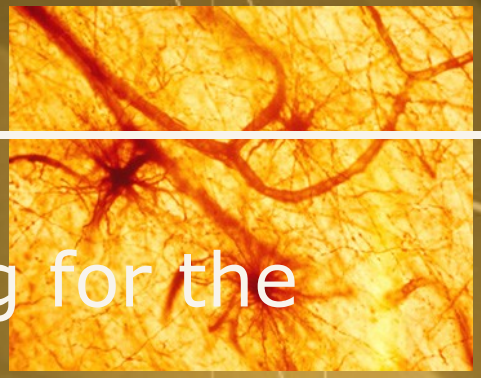
Synthetic Polymers



- Have previously been used with other cell types for many applications including tissue engineering and controlled drug delivery.
- Optimizing these materials may lead to the development of reproducible, scalable, nontoxic, and nonimmunogenic materials for in vitro expansion or differentiation, as well as in vivo implantation, of NSCs.
- Fully synthetic, biofunctionalized materials can support cell proliferation, and the addition of differentiating media leads to multipotent differentiation.
- Future work may explore the extent to which the substrate can guide cell lineage commitment.



Conclusions



- Neural stem cells are promising for the treatment of CNS injuries.
- Engineered materials containing natural and/or synthetic components can support the expansion and potentially in the future induce the lineage-specific differentiation of NSCs in vitro.