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TISSUE ENGINEERING
III. Growth Factors and Genes

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DIFFUSIBLE REGULATORS OF CELL FUNCTION

Cytokines are polypeptides (proteins) that regulate many cell functions. They act on a target cell by binding to specific high-affinity receptors. Cytokines that act on the same cell that produced them are called **autocrine** factors; those that act on other cells are called **paracrine** factors; those that act systemically (through the vascular system) are referred to as **endocrine** factors. Molecules that switch on (*i.e.*, regulate) mitosis are referred to as **growth factors**.

DIFFUSIBLE REGULATORS OF CELL FUNCTION

Eicosanoids are chemically related signaling lipid molecules made primarily from arachidonic acid (fatty acid). Eicosanoids include **prostaglandins**, **leukotrienes**, **thromboxanes**, and **lipoxins**. Prostaglandins are continuously synthesized in membranes from precursors (20-carbon fatty acid chains that contain at least 3 double bonds, *e.g.*, arachidonic acid) cleaved from membrane phospholipids by phospholipases, membrane-bound enzymes. They are continuously released by the cell, and are degraded by enzymes in the extracellular fluids. The subscript of PGE2 refers to the 2 double bonds outside the ring structure.

DIFFUSIBLE REGULATORS OF CELL FUNCTION

Cytokines

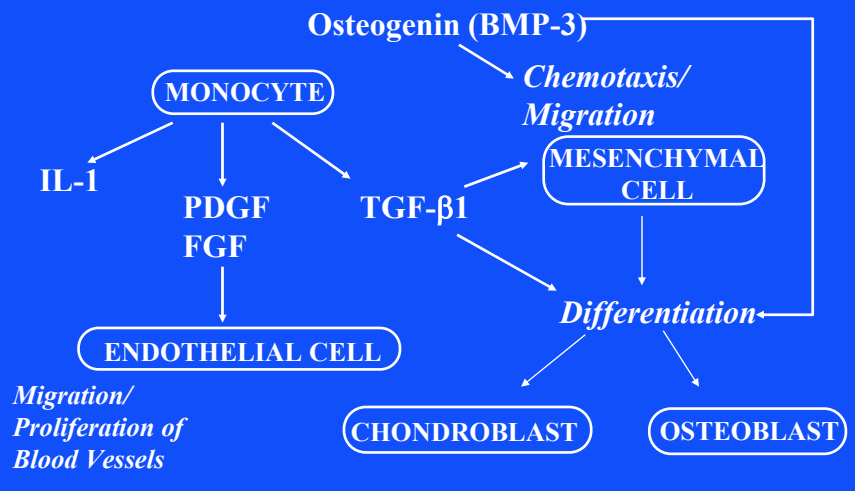
- Interleukins
  - IL-1
  - IL-6
- Tumor Necrosis Factor (TNF)
- Platelet Derived Growth Factor (PDGF)

- Insulin-like Growth Factor (IGF)
  - IGF-1 and IGF-2
- Fibroblast Growth Factor (FGF)
  - basic FGF (FGF-2)
- Transforming Growth Factor (TGF)
  - TGF-β

BONE MORPHOGENETIC PROTEINS

- Induces bone formation in nonosseous tissue
- Sources: bone matrix, tooth matrix, osteosarcoma, epithelia
- Heterodimers of BMP more active than homodimers (BMP-2/7 is 20 times more active than BMP-2)

BMP / TGF ACTIVITY



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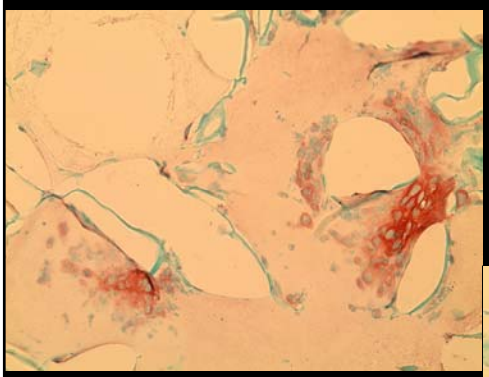
GENE-SUPPLEMENTED COLLAGEN-GAG MATRICES

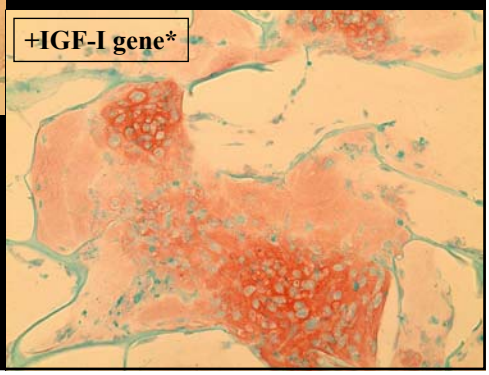
- Bolus delivery of growth factors to a defect does not allow for a prolonged effect.
- Transfer of the gene for a selected cytokine to the cells involved in the reparative process may maintain therapeutic levels through the later phases of the repair process.
- Prolonged release is necessary when
  - target cells do not appear at the implant site until days or weeks postoperative
  - there is a premature loss of expression in transfected cells
  - transfected cells migrate away from the defect site.

P2 Canine Chondrocyte-Seeded Type II Collagen (CD x-linked), 4w



* Cells in the collagen scaffold transfected with the gene for IGF I using Geneporter

+IGF-I gene*



Samuel RE, Delivery of plasmid DNA to articular chondrocytes via novel collagen-glycosaminoglycan matrices. Human Gene Therapy 2002;13:791 802

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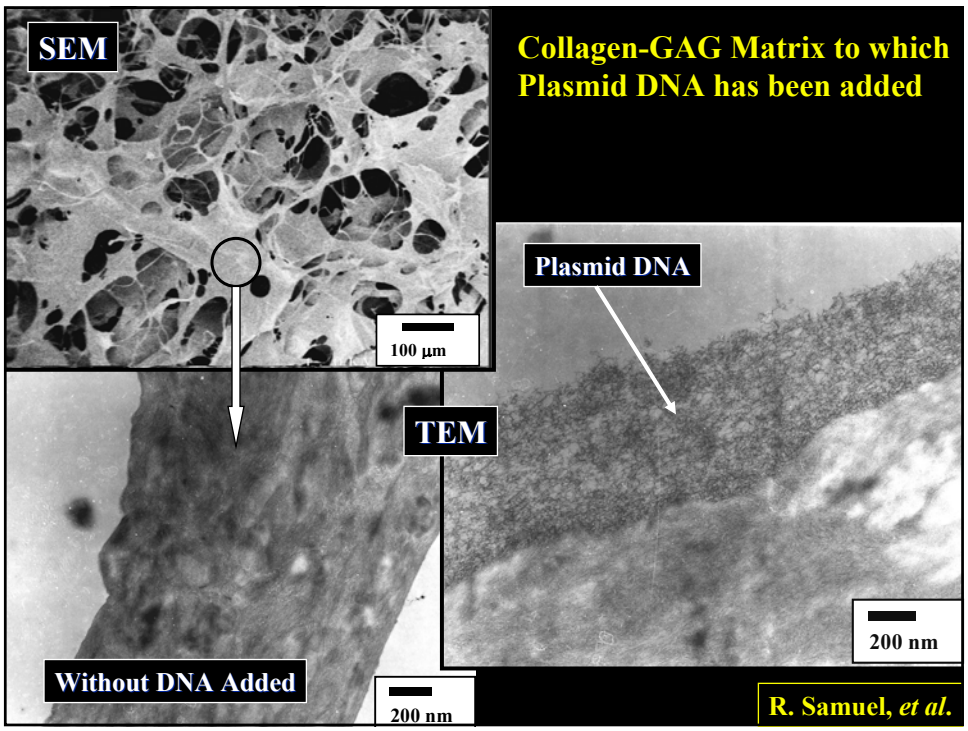
GENE-SUPPLEMENTED COLLAGEN-GAG MATRICES

- Plasmid DNA added to pre-fabricated collagen-GAG matrices can transfect seeded chondrocytes.
- Conditions under which the DNA is incorporated into the matrices will affect retention and prolonged release
- Cross-linking method will affect transfection rates



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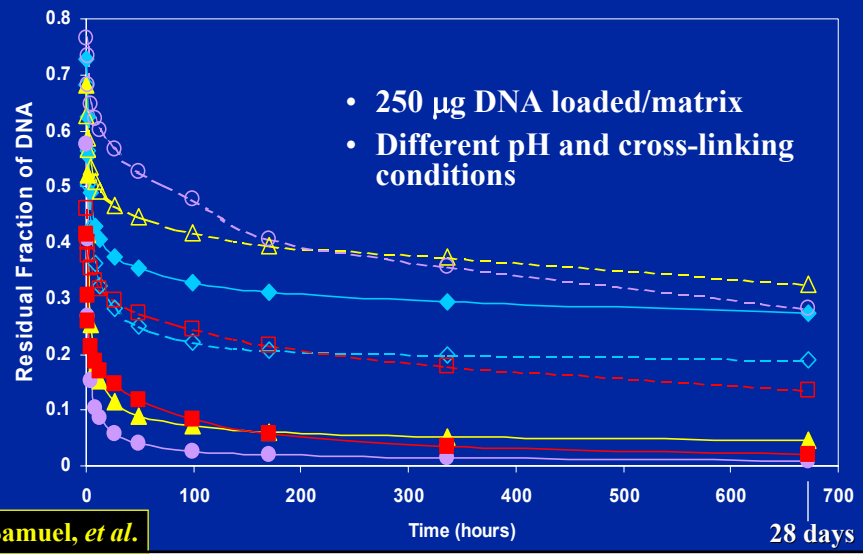
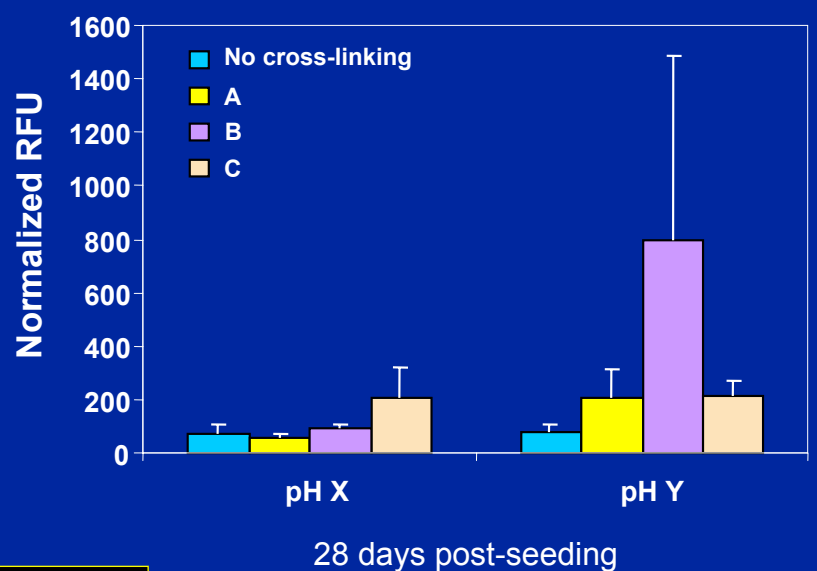
Retention of DNA in Collagen Matrices

Luciferase gene --> DNA + matrix + adult canine chondrocytes



R. Samuel, *et al.*

In Situ Transfection of Chondrocytes Based on Luciferase Activity



28 days post-seeding

R. Samuel, *et al.*

DISCUSSION

- Plasmid DNA could be bound to pre-fabricated collagen-GAG matrices
- Small percentage of DNA was tightly bound, higher in matrices prepared at a certain pH
- Higher level of transfection in matrices prepared at other pHs

DISCUSSION

- Selected collagen-GAG matrices could be formulated to provide for the prolonged (greater than 1 month) release of plasmid DNA.
- A significant percentage (20-40%) of the DNA added to the matrices resist passive release into the leaching buffer. For comparison, in a prior study (Shea, *et al.*, Nature Biotech., 1999) investigating release of plasmid DNA from copolymers of D,L-lactide and glycolide, less than 10% of the DNA remained in the synthetic polymer construct after 28 days in leaching studies.