C. Synthesis of biologically active scaffolds (regeneration templates)

1. History of biologically active scaffolds (regeneration templates).
2. Physical chemistry of collagen: Melting of collagen quaternary structure (thromboresistance). Melting of collagen tertiary structure (gelatinization).
3. Synthesis of ECM analogs: Ionic complexation collagen/GAG, formation of pore structure, crosslinking.
4. Biological activity of ECM analogs depends critically on structure.

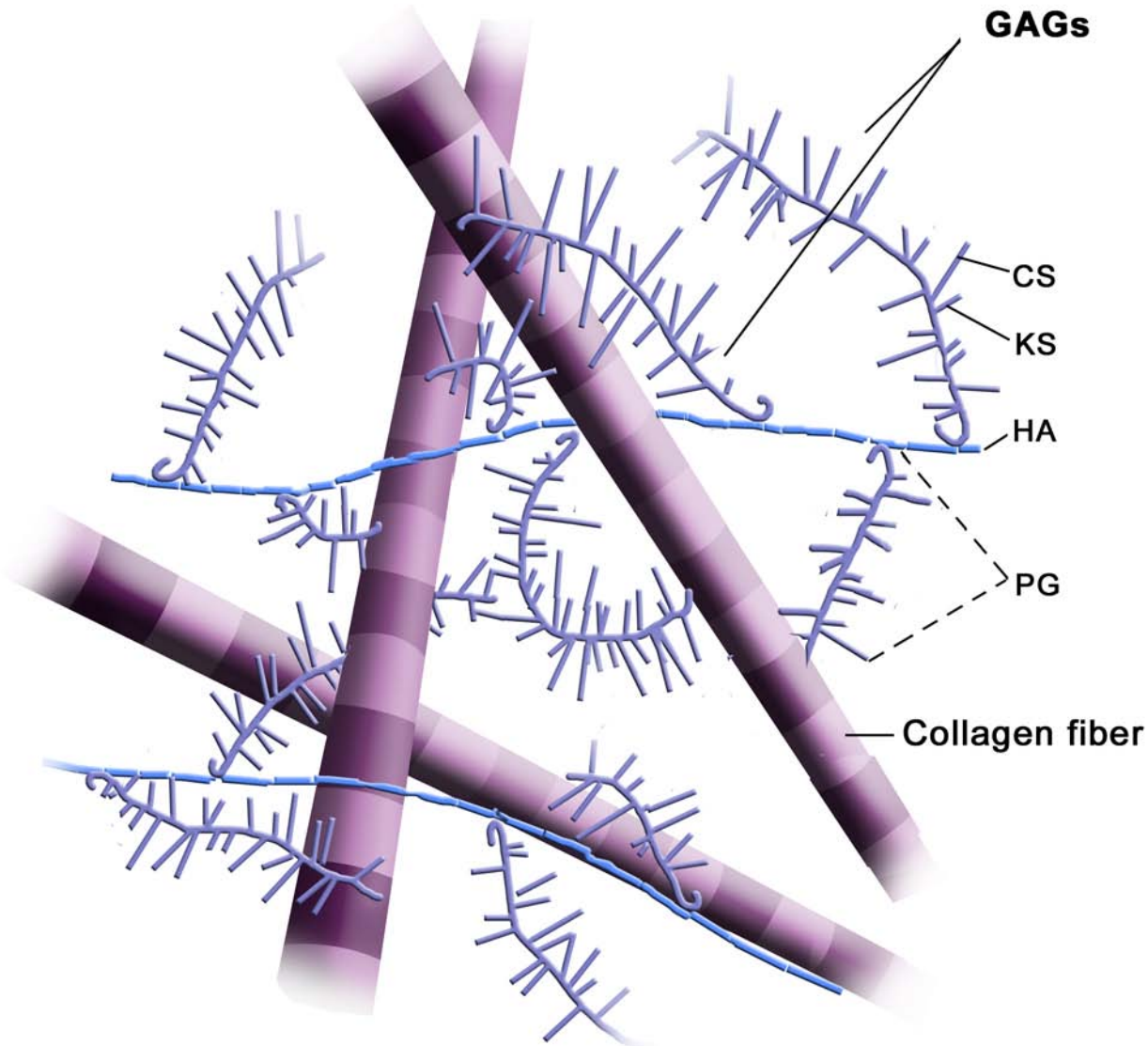
1. History of biologically active scaffolds (regeneration templates).

- **1974-75 Synthesis and characterization of the first biologically active scaffolds. Scaffolds defined as very highly porous polymeric constructs that are commonly used, either unseeded or seeded with cells, to synthesize tissues and organs in vitro or in vivo (Yannas et al., 1975a,b,c; 1979; 1980a,b,c).**
- **1979-80 First clinical use of a biologically active scaffold to regenerate the dermis (treatment of massively burned children) (Burke et al., 1981).**

1. History of biologically active scaffolds (regeneration templates). (continued)

- 1981-82 Implantation (grafting) of a cell-seeded scaffold. Keratinocyte-seeded template regenerates simultaneously dermis and epidermis in animals (Yannas et al., 1982).
- 1985 Regeneration of peripheral nerves across unprecedented distances in animals using a biologically active scaffold (Yannas et al., 1985).
- 1989 Identification of structural features that account for template regenerative activity (Yannas et al., 1989).
- 1996 FDA approves first scaffold (Integra) for treatment of burned patients and, later, for plastic and reconstructive surgery of skin (2001).

Analogs of extracellular matrix



2. Physical chemistry of collagen:

--- Melting of collagen tertiary structure: acceleration of biodegradation rate.

--- Melting of collagen quaternary structure: thromboresistance.

COLLAGEN STRUCTURE

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Primary structure (amino acid sequence) of Type I collagen

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Mechanical (viscoelastic) behavior of colagen and gelatin. Proteins were Progressively diluted with glycerol to elicit the entire spectrum of their viscoelastic behavior. Gelatin shows a rubberlike state. Collagen does not.

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Degradation of collagen fibers by collagenase

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Degradation of collagen molecule by collagenase to gelatin. Gelatin itself degrades much faster than collagen.

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Melting of quaternary structure of collagen fibers occurs below pH 4.5. Melting confers thromboresistance to the scaffold. Platelets Do not aggregate unless the quaternary structure Is intact. Blocking of platelet aggregation leads to downregulation of the inflammatory response at the site of grafting or implantation.

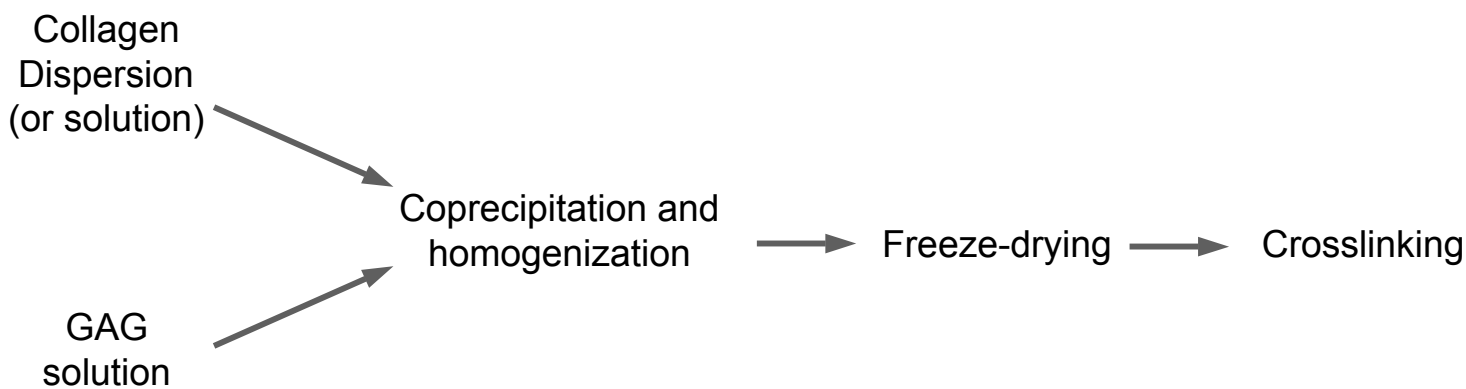
3. Synthesis of active ECM analogs:

--- Ionic complexation of collagen/GAG.

--- Formation of pore structure.

--- Crosslinking.

Collagen-GAG membrane formation process



Glycosamino-glycans

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Diagram of Chondroitin 4-Sulfate

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Diagram of Dermatan Sulfate.

disaccharide repeat unit

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Diagram of Heparan Sulfate.

Biologically active collagen/GAG scaffold (dermis regeneration template)

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Procedures used to study the pore structure of scaffolds. Unlike collagen sponges (used as hemostatic agents), regeneration templates have very high pore volume fraction. typically >95%.

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Crosslinking binds GAG covalently to collagen to produce a graft copolymer. Solvents with high ionic strength fail to separate the two polymers from each other.

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4. Biological activity of ECM analogs depends critically on structure.

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Which ECM analogs are biologically active as regeneration templates?

Critical Structural Feature	Role in regeneration
A. SKIN	
Chem. Composition >2% GAG Deleted collagen quaternary structure	Ligand identity Downregulation of inflammatory response
Pore diameter 20—120 μm	Ligand density
Degradation half-life 10-15 d	Duration of ligands
B. NERVE	
Chem. Composition Deleted collagen quaternary structure	[not studied] [not studied]
Pore diameter ~ 5 μm	Ligand density
Degradation half-life ~ 1-10 wk	Duration of ligands

Conclusions

1. Certain ECM analogs are biologically active scaffolds (regeneration templates) that induce regeneration of tissues and organs: skin, peripheral nerve and the conjunctiva (eye) in humans and experimental animals.
2. Regeneration templates lose their activity if the following structural features fall outside a narrow range: chemical composition, collagen quaternary structure, pore diameter, degradation rate.
3. The data suggest that templates induce regeneration in a defect by blocking selectively the contraction process that leads to closure of the defect in adults.
4. Templates block contraction by two basic mechanisms. First, by downregulating differentiation of fibroblasts to myofibroblasts. Second, by binding most of the contractile cells in the defect over a period corresponding to the duration of contraction in that defect. Binding requires the presence of appropriate ligands (chem. composition) at a minimal density (pore diameter) over a critical duration (degradation rate).