Cartilage

Tipi di cartilagine

- Ialina
- Elastica
- Fibrocartilagine

- Articolare
- trachea

Cartilagine articolare

- 1-7 mm
- Matrice amorfa:
  - H20 (60%), proteoglicani (4-7%), acido ialuronico
  - collagene tipo II (15-22%) et al (<2%)
- Continuamente sottoposta a compressione
- Non vascolarizzata (nutrizione x diffusione)
- Possibilità di danni (traumi, degenerazione progressiva)
- Minima o nulla capacità di rigenerarsi

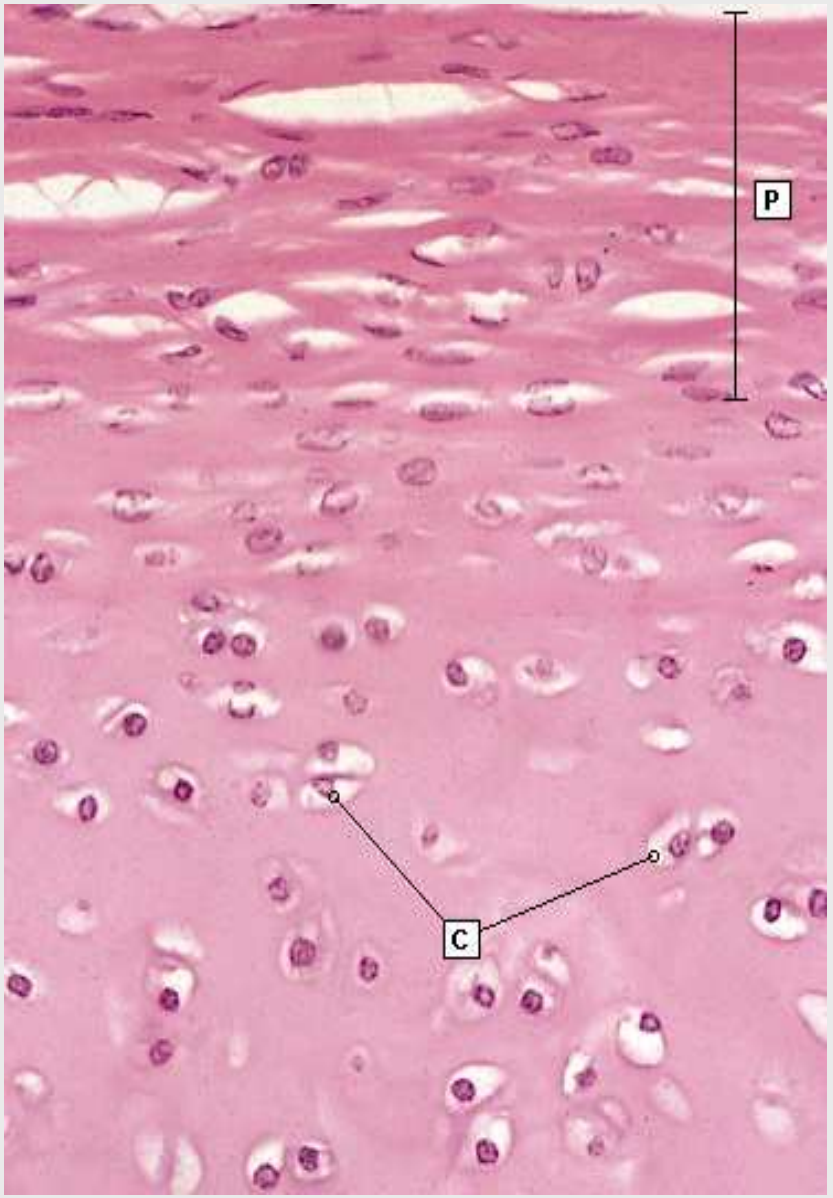
Mantero et al. 2009

Cartilagine ialina

gruppi isogeni

tessuto semi-trasparente di colore grigio-bluastro
(dal greco hyalos = vetro)

Anelli tracheali, della laringe e del setto nasale
Superfici articolari delle sinovie (es. ginocchio)
Estremità delle coste che si connettono con lo sterno

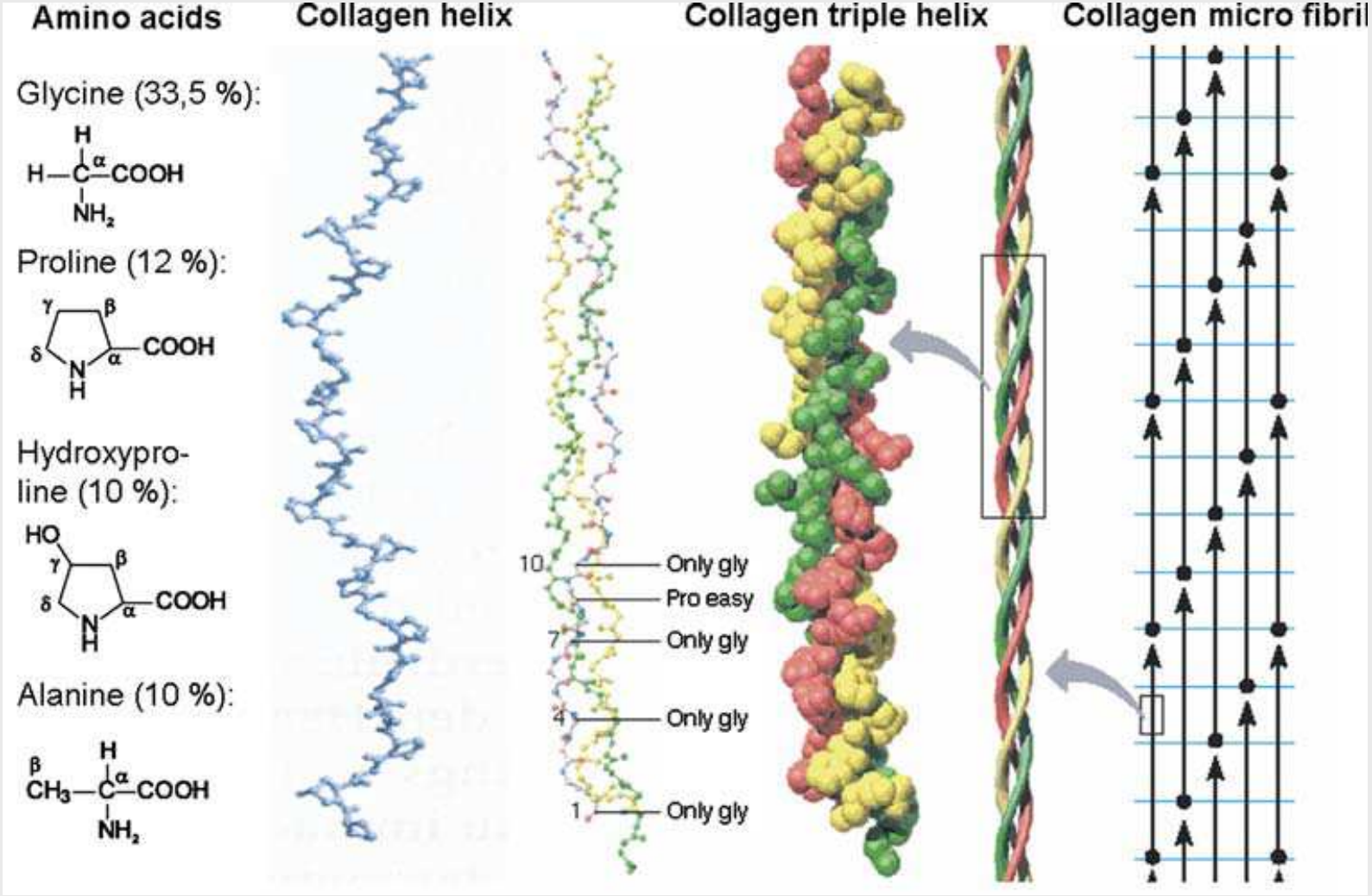


Cartilagine

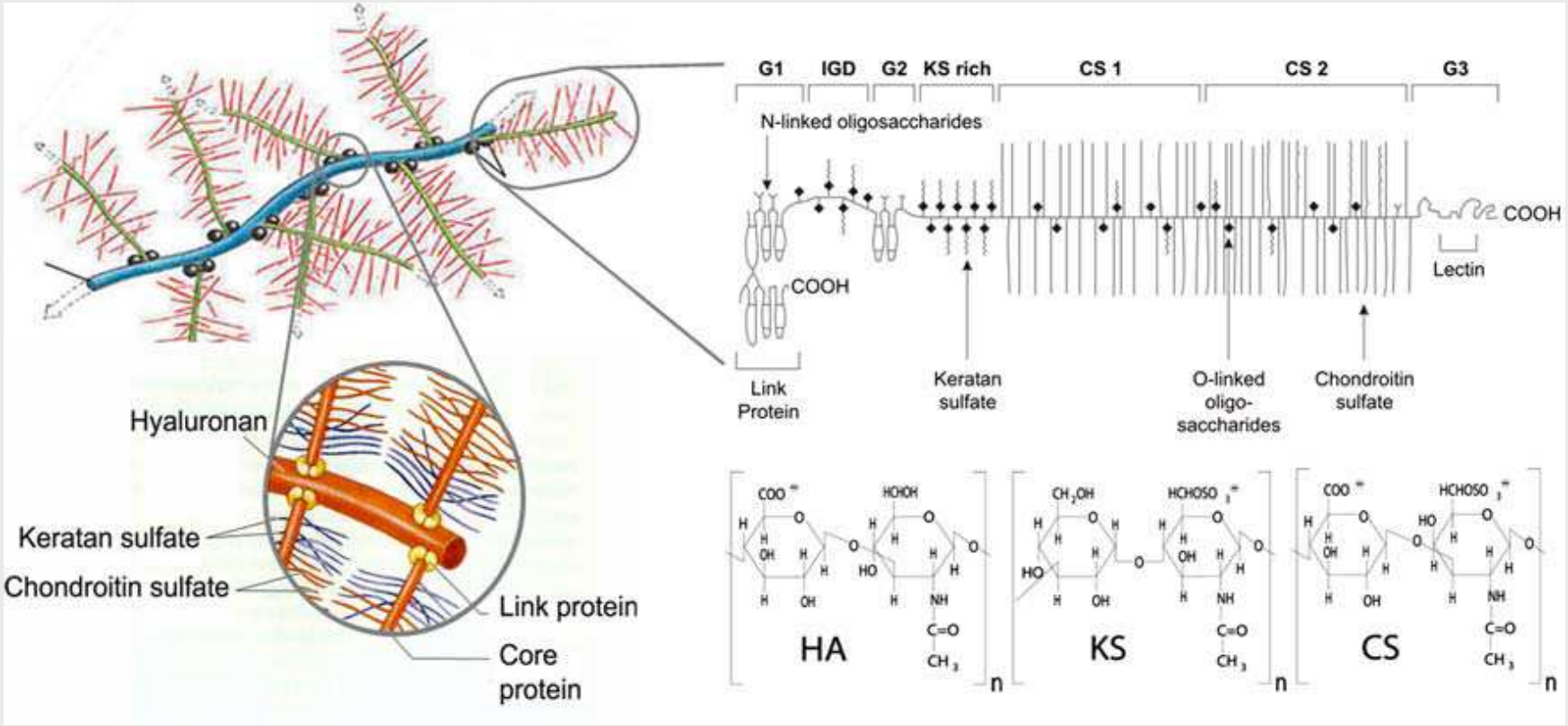
- matrice extracellulare:
  - fibre collagene tipo I, tipo II, IX- X –XI.
  - fibre elastiche,
  - SOSTANZA FONDAMENTALE : glicoproteine, proteoglicani. I GAG sono per lo più condroitin-solfato e cheratan-solfato, ac. ialuronico
  - Aggrecano e' un proteoglicano caratteristico della cartilagine
  - acqua 70-80 % del peso secco del tessuto

  → incomprimibilità

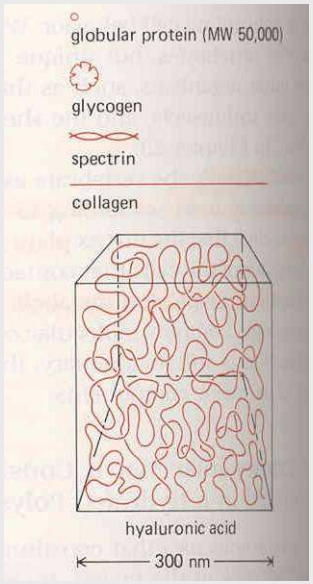
Il collagene

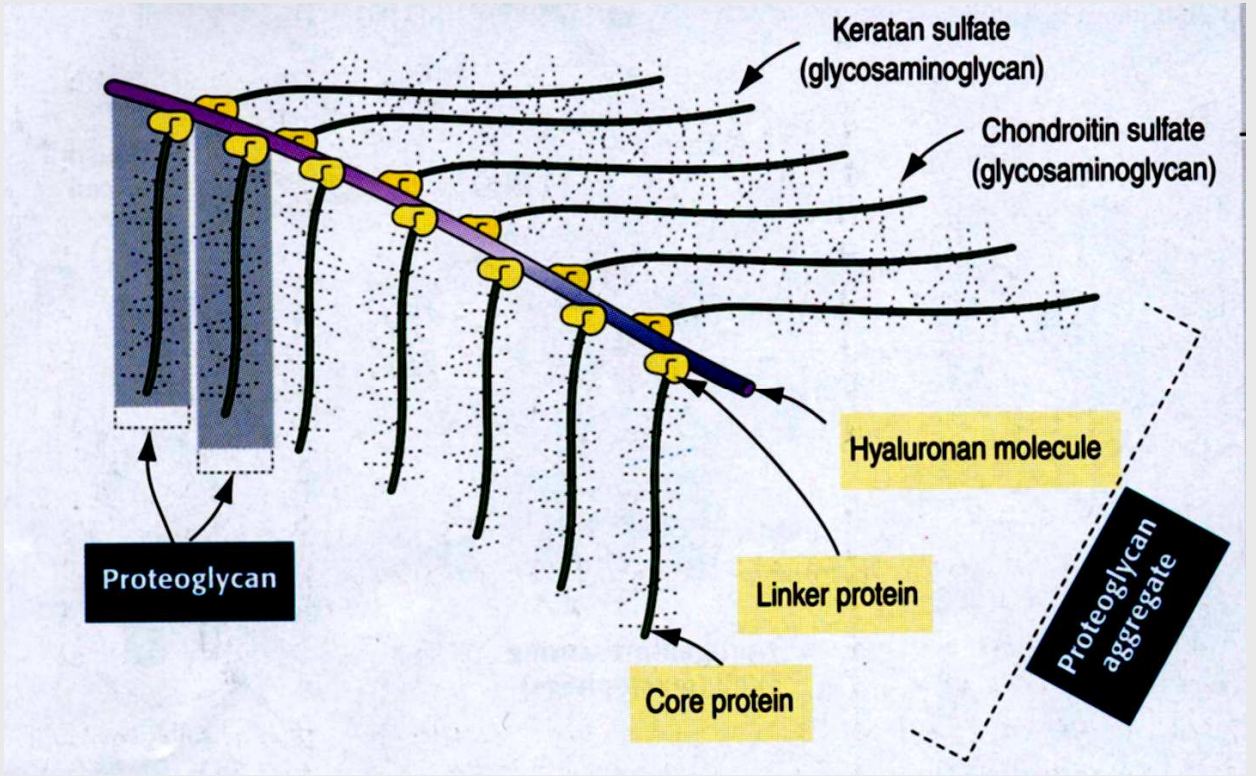


Proteoglicani della cartilagine articolare

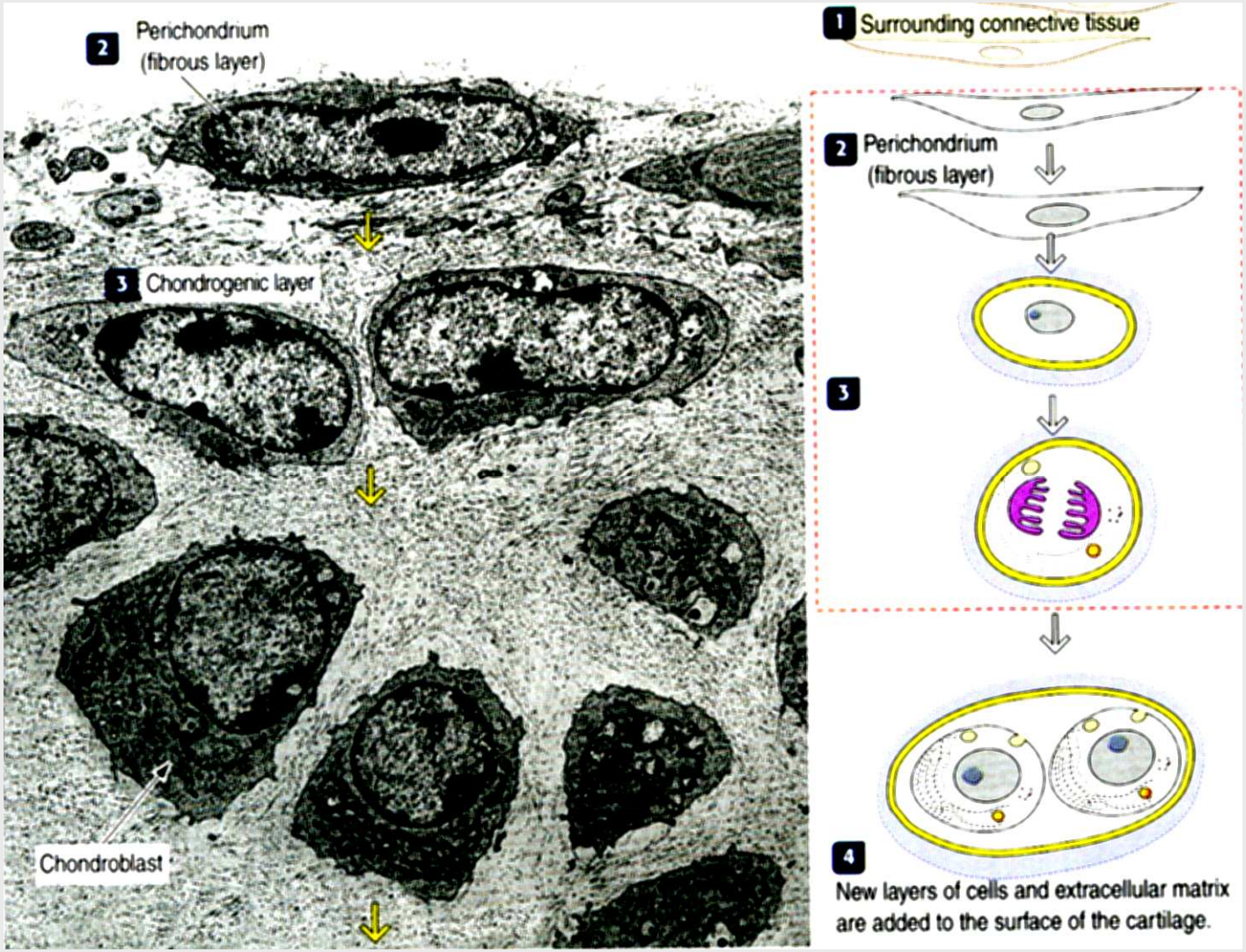


Struttura dell'aggrecano



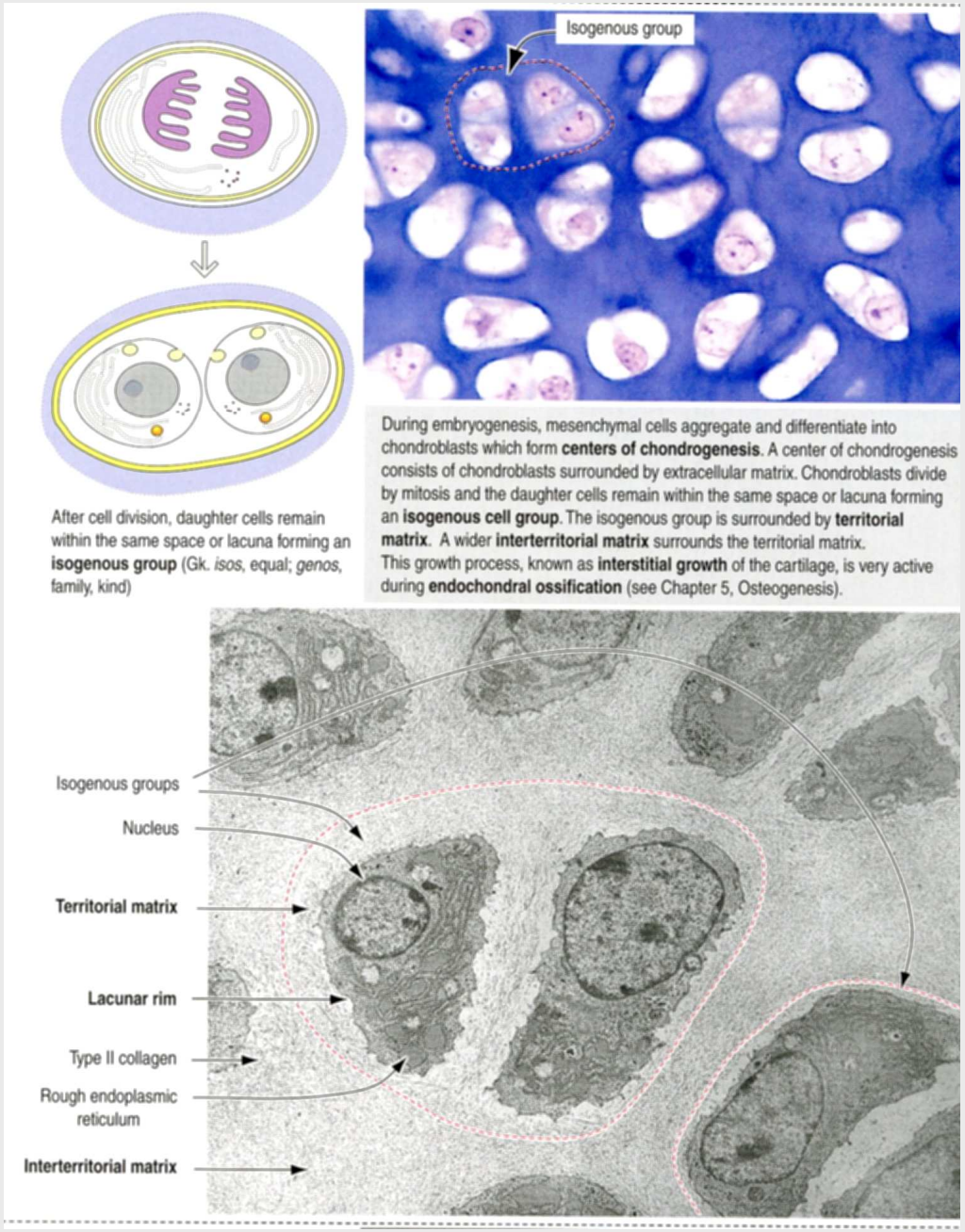


Crescita apposizionale



Crescita interstiziale

→in entrambi i casi: limitata capacità rigenerativa
→ problema medico



Growth factors in chondrogenesis

- TGF-β (5 proteins) & BMP (20 members)
- FGF: 22 x 4 receptors
- IGF-1: 2 members x 2 receptors
- Wingless family: >20 members, Fzd receptor
- Hedgehog family: 3 members

Trends in Biotechnology Vol.27 No.5

TGF-β family in chondrogenesis

- TGF-β family: bone morphogenetic proteins (BMPs), activins and inhibins
  - Type II receptor → type I receptor →Smads
  - Direct injection: osteophyte formation, swelling and synovial hyperplasia
- → tightly coordinated regulation of TGF-β is needed to control chondrogenesis.

- The use of BMP-2, - 4 and -7 approved for some clinical applications, but their potential to enhance cartilage repair still needs to be validated in humans

FGF family in chondrogenesis

- Activated ERK1/2, p38 and JNK, PI3K PLC pathways
- Many dysplasias attributed to specific mutations in the genes encoding the FGFR1, -2 and -3
- forced expression of FGFR3 in the murine MSC line sufficient for chondrogenic differentiation
- FGF18 (ligand of FGFR3) promoted the differentiation of limb bud mesenchymal cells to produce cartilage matrix
- in a rabbit model, FGF2 stimulated articular cartilage
- restoration in temporomandibular or articular cartilage defects

IGF family in chondrogenesis

- IGF-1 (cartilage repair), 2 (development) & 2 receptors
- At least 6 IGF-binding proteins (IGFBPs) and multiple proteases
- In embryonic development, mice with IGF-1-/- mutations: severe growth retardation and have developmental defects in various organs.
- In adults, IGF-1 and IGF1R are expressed by chondrocytes, osteoblastsand osteoclasts
- IGF-1 considered an essential mediator of cartilage homeostasis (stimulates proteoglycan synthesis, promotes chondrocyte survival and proliferation
- IGF-1 induces the MSC differentiation, chondrocyte migration
- IGF-1 + chondrocytes induce improve repaired cartilage consistency (horse)

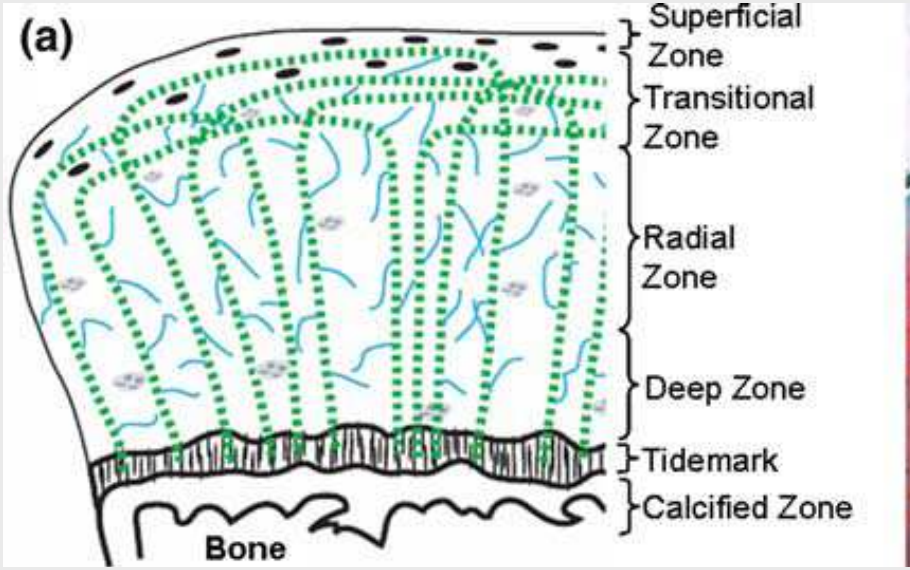
Wingless family in chondrogenesis

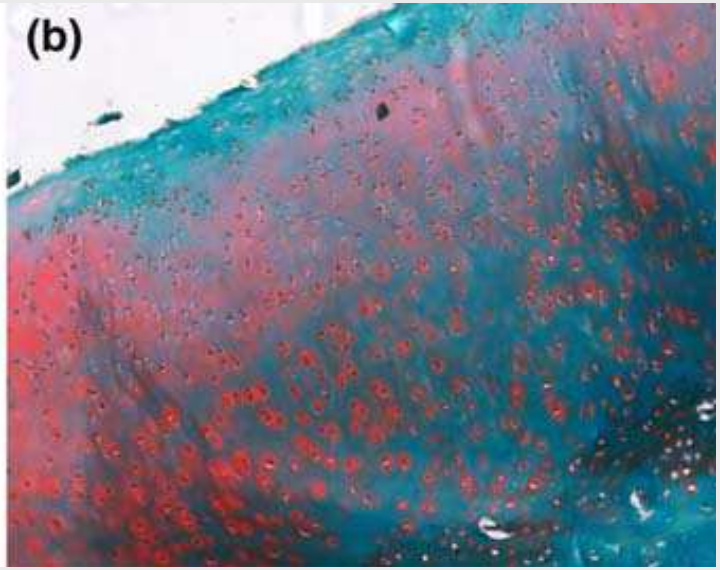
- > 20 members, Frizzled receptor
  β-catenin-dependent pathway, PKC, PLC, JNK
- involved in both early and late skeletal development
- Wnt-1, Wnt-4, Wnt-7a and Wnt-8 block chondrogenic differentiation
- Wnt-5a, Wnt-5b and Wnt-11 regulate chondrocyte proliferation and hypertrophic maturation
- **dual roles in cartilage**
- (i) it is an important regulator of chondrocyte development
- (ii) deregulation of Wnt signalling might lead to disease, in particular to osteoarthritis

Hedgehog family in chondrogenesis

- 3 members: Sonic, Indian, Desert hh
- Hh → Patched →Smoothened (master regulator of transcription factors Gli family)
- Indian hh → PTHrP → prevents chondro diff (mutants have << chondro prolif

Cartilagine articolare

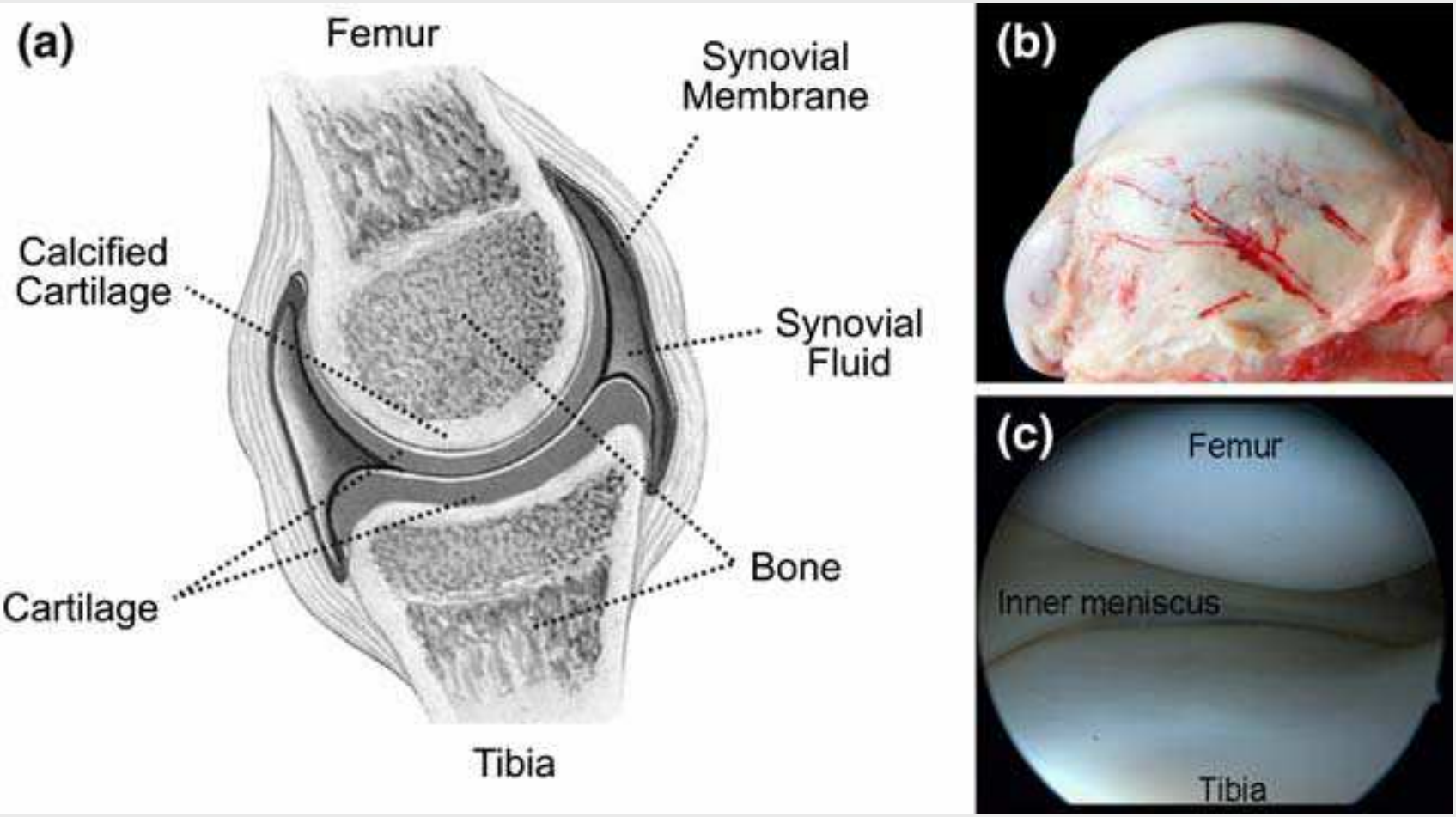




Proteoglicani
Fibrille
cellule

Osso in verde
Cartilagine in rosso (PG)
Cartilagine ossificata in rosa

Schema di articolazione del ginocchio



Eur Biophys J (2007) 36:539–568

Trapianto di cartilagine autologa prodotta ex vivo

area danneggiata

aggiunta di strato di fibrina

inserzione pezzo prodotto ex vivo

Rigenerazione in vitro

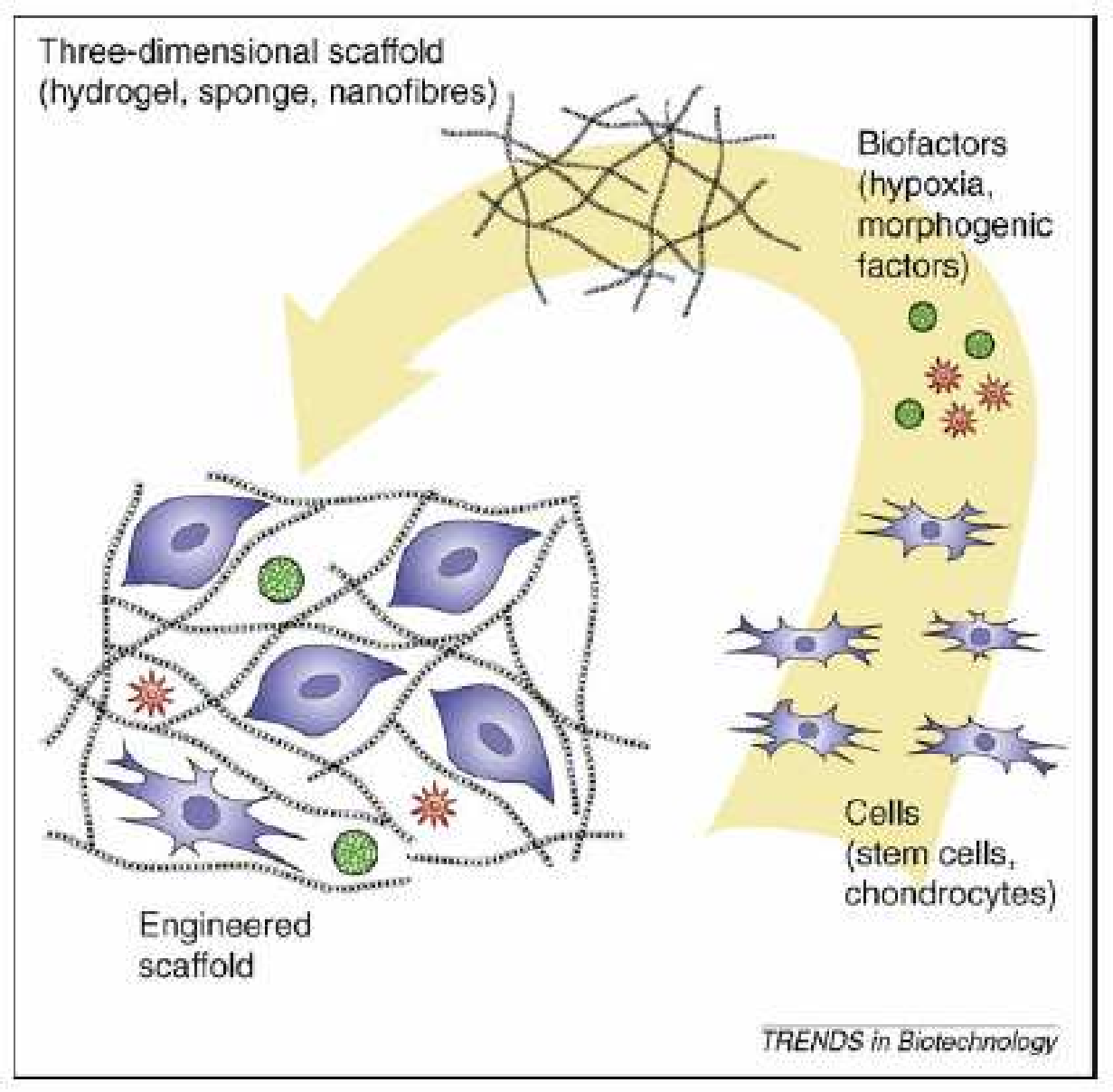
- Autotrapianto
  - Espansione in vitro
  - Integrazione con i tessuti circostanti
- Matrice sintetica 3D, alta porosità, trama filamentosa, biodegradabile

- Tortelli F, Cancedda R. Three-dimensional cultures of osteogenic andchondrogenic cells: a tissue engineering approach to mimic bone and cartilage in vitro. Eur Cell Mater. 2009 Jun 30;17:1-14. Review.
- Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noël D. Cartilageengineering: a crucial combination of cells, biomaterials and biofactors. Trends Biotechnol. 2009 May;27(5):307-14. Review.
- Schulz, R.M. and Bader, A. Cartilage tissue engineering and bioreactor systems for the cultivation and stimulation of chondrocytes. Eur. Biophys. J. 2007; 36: 539–568

Problem in TE

- identifying additional specific growth factors and evaluating the optimal combination of cells, growth factors and scaffolds that is able to respond to the functional demand placed upon cartilage tissue replacement in clinics.

Cells, biofactors and scaffolds for cartilage formation



Storia

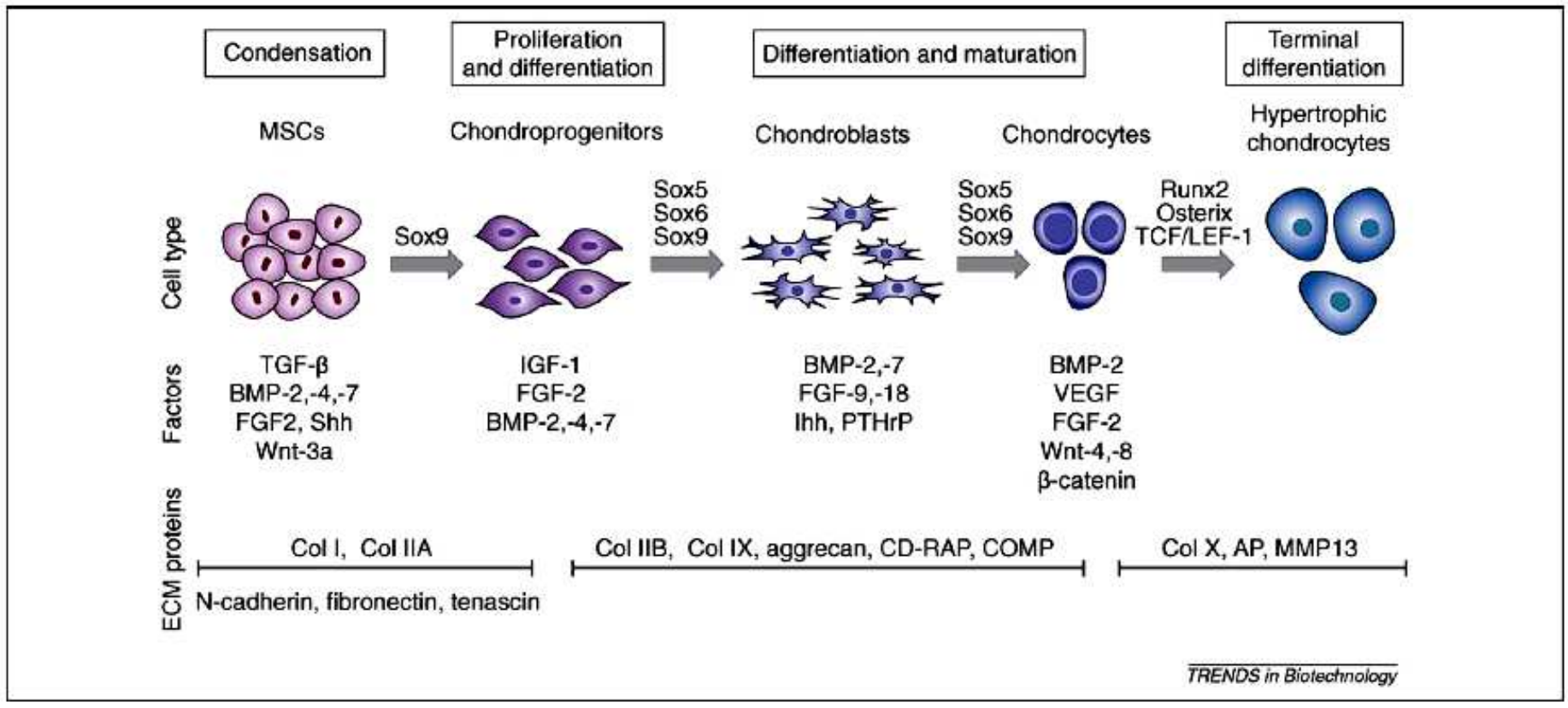
- mechanical penetration of the sub-chondral bone to stimulate marrow entry inside the lesion, on periosteum and perichondrium grafts or on autologous chondrocyte transplantation

  → no good results
- latest strategies rely on cell-based therapies, which are currently in the developmental or preclinical stages, and involve biomaterials that have been seeded with chondrocytes or progenitor cells and/or chondrogenic factors.

Source of cells

- CHONDROCYTES (also from nasal cartilage): these cells suffer from two major concerns:
  - instability in monolayer culture → dedifferentiate in 2D
  - the rareness of the donor tissue
- mesenchymal stem cells (MSC)
  - CD73+, CD90+ , CD105+, CD14– or CD11b–, CD19– or CD79a–, CD45– and HLA-DR–;
  - Immunoregulatory (escape immune recognition and inhibit the host defence mechanisms
- -

GF leading to the differentiation of mesenchymal stem cells (MSCs) towards chondrocytes.



Main biomaterials used for the 3D culture and transplantation of chondrogenic cells in cartilage TE

Matrices		Cells	
Type	**Material**	**Chondrocytes**	**MSCs**
Protein-based	Collagen	[18]	[20]
	Fibrin	[81]	[82]
Polysaccharide-based	Alginate	[83]	[86]
	Chitosan	[84]	N/A
	Hyaluronic acid	[21]	[87]
	Cellulose	[85]	N/A
Synthetic	PLGA (poly[lactic-co-glycolic acid])	[88]	N/A
	PLA (polylactic acid)	N/A	[90]
	PEG (polyethylene glycol)	[89]	[91]

Trends in Biotechnology, 2007; 27 (5)

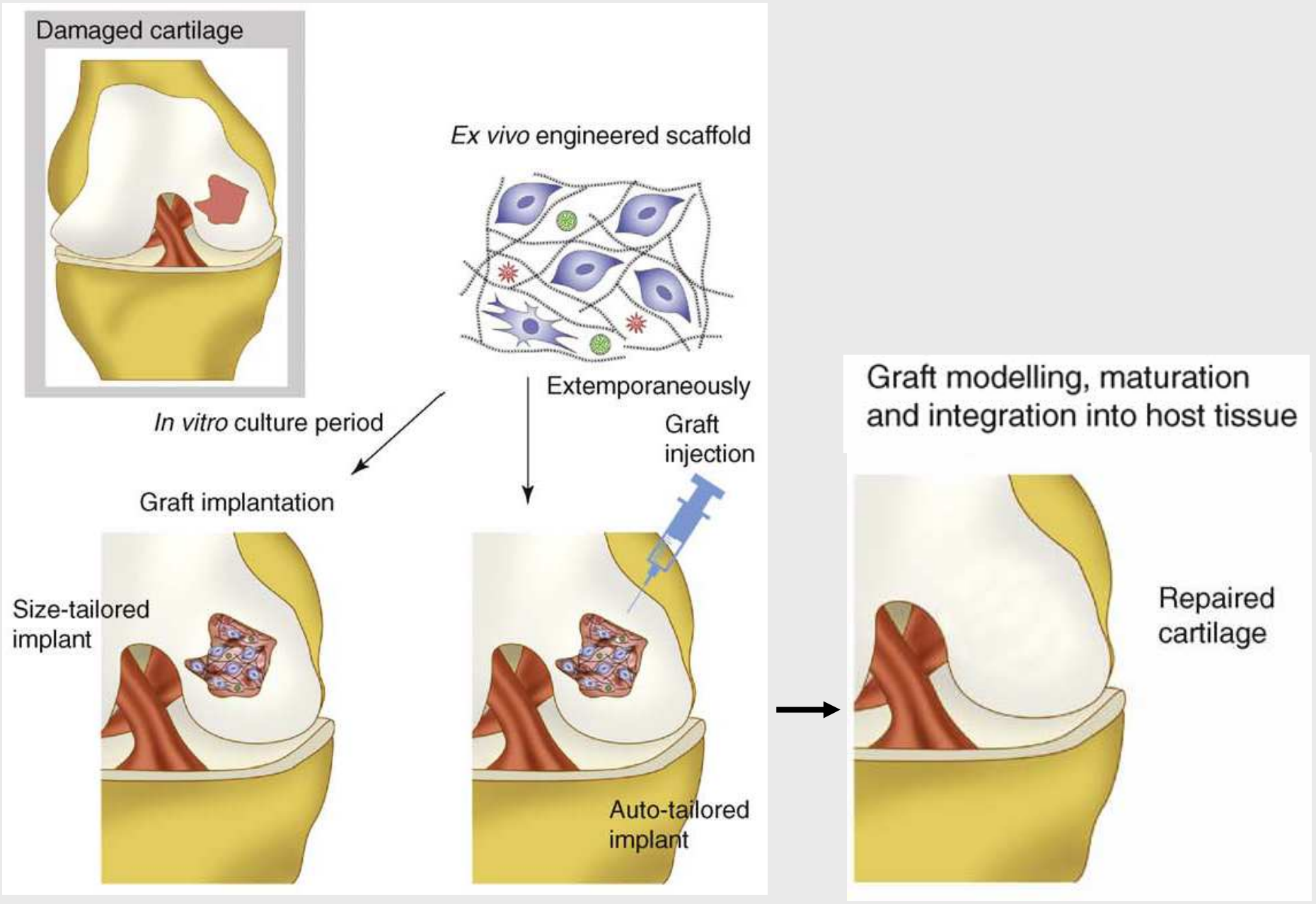
Matrici

- USA: collagene
- EU:
  - polimeri artificiali (PGA-PLA)
  - acido ialuronico: Fidia: HYAFF: microfibre 13 μm, stirate e rilassate, attorcigliate, tagliate e cardate → foglietti quadrati cm x 2-5 mm h
- Su queste: semina cellule in condizioni dinamiche x migliore distribuzione
  - Fiasche rotanti
  - bioreattori: try to mimic the mechanical loading experienced by cartilage in vivo which results from the motion of and forces on joints

Bioreattore

- Circuito di perfusione
  - Pompa peristaltica
  - Sistema di ossigenzione
  - Reservoir
- Sistema chiuso (giunti e guarnizioni)
  - → sterilità
-

- Despite encouraging clinical results, the above-mentioned matrices suffer a major limitation in that that they all require a surgical incision into the joint to be implanted. In this context, the development of injectable biomaterials that are suitable for mini-invasive transplantation of chondrogenic cells remains challenging for researchers.


Damaged cartilage
Ex vivo engineered scaffold
Extemporaneously
In vitro culture period
Graft injection
Graft implantation
Size-tailored implant
Auto-tailored implant
Graft modelling, maturation and integration into host tissue
Repaired cartilage

Hydrogel

Hydrogels

- a new class of biomaterials that could potentially be injected transcutaneously into joints.
- composed of a viscous polymer made of synthetic or natural hydrophilic macromolecules, which are able to form a hydrogel after physical, ionic or covalent crosslinking
- high water content close to that found in cartilage and therefore mimicking the 3D environment of cells in cartilage

Hydrogels

- three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids commonly used to mimic the chondrogenic environment. There are two main classes of
- hydrogels: i) naturally derived hydrogels, such as collagen and alginate
- ii) synthetic-based hydrogel

- in bovine articular chondrocytes grown in collagen sponges to which hydrostatic fluid pressure (HFP) was applied, an enhancement of the synthesis of cartilage-specific matrix components has been reported

Natural polysaccharide (Alginate)

- alginate, chitosan and hyaluronic acid have been also explored for chondrocytes encapsulation.
- alginate is a natural, non-mammalian polysaccharide that forms a gel in the presence of divalent cations by means of ionic crosslinking; it does not degrade, but rather dissolves when the divalent cations are replaced by monovalent ions
- + RGD → better cell adhesion

Natural polysaccharide (chitosan)

- made by partially deacetylated chitin
- high-degrees of deacetylation lead to slower degradation times but better cell adhesion due to increased hydrophobicity
- cationic nature → facilitate the entrapment of the highly anionic aggrecan produced by chondrocytes
- Used with success for nucleus pulposus

Other materials used for scaffolds

- silk fibroin hydrogels
- PEG

biomaterials, composed of spontaneously self-assembling peptides

- have amino acid sequences of alternating hydrophobic and hydrophilic side groups
- form stable β-sheet structures when dissolved in deionised water
- have a nanofibre structure smaller than other polymer microfibres and present a unique polymer structure with which cells may interact
- KLD-12 as a 3D scaffold for encapsulation of bovine chondrocytes
- cultured in static conditions up to 26 days synthesized and accumulated a cartilage-like ECM

Stimuli involved in condrogenesis

- HYPOXIA
- Mechanical stimuli

    to be considered when expanding cells in vitro and building bioreactors

Hypoxia in cartilage is normal and is considered a **stimulus**

- oxygen concentration in the articular cartilage is low (1 to 7%) [HIF-1α and β]
- Hypoxia shown to increase the synthesis of ECM proteins in cultured chondrocytes in vitro
- low oxygen tension is a key regulatory factor of proliferation, differentiation and activity of chondrogenic cells
- inhibits the expression of type X collagen (major marker of chondrocyte hypertrophy during the chondrogenesis of epiphyseal chondrocytes and of adipose-derived MSCs), thereby preventing the potential calcification of engineered cartilage.

Mechanical stimuli

- hydrostatic pressure
- compressive straint
- shear straint
- Physiological loading is a pivotal factor influencing the chondrogenic differentiation of MSCs during articular cartilage development.
- Moreover, mechanical stimuli on chondrocytes reported to be essential for the maintenance of cartilage integrity
- the above-cited parameters have been integrated into bioreactors

Majors obstacles to persistent regeneration of cartilage

- leakage of the cell suspension, loss of regenerative cells through cell death after transplantation
  - necrosis, apoptosis, inflammatory cytokines, metalloproteinases, nitric oxide, serum deprivation or mechanical forces
- Insufficient capacity of chondrogenic cells to integrate within surrounding tissue
  - insufficient secretion of matrix proteins by implanted cells
  - tissue complexity
- Induction of dedifferentiated chondrogenic cells by inflammatory stress
  - Instability of implanted cells (→ fibrocartilage)

How is today and what next

- feasibility of such approaches for cartilage repair
- acceptable clinical results

  but

- still far from being able to generate a tissue that is comparable to native cartilage with respect to quality and stability
- require a complete integration of the neocartilage and reconstitution of an appropriate zonal organization
- Gene therapy?

Articolo suggerito

JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2010; **4**: 25–29.
Published online 15 October 2009 in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/term.205

RESEARCH ARTICLE

A nanofibrous cell-seeded hydrogel promotes integration in a cartilage gap model

S. A. Maher[1*], R. L. Mauck[2#], L. Rackwitz[2##] and R. S. Tuan[2]