Biomedical Materials

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What advantages do young scientists have?

- Innovative ideas: seeing without prejudice

  ↓ Imagination (lateral thinking)

- Modern methodology

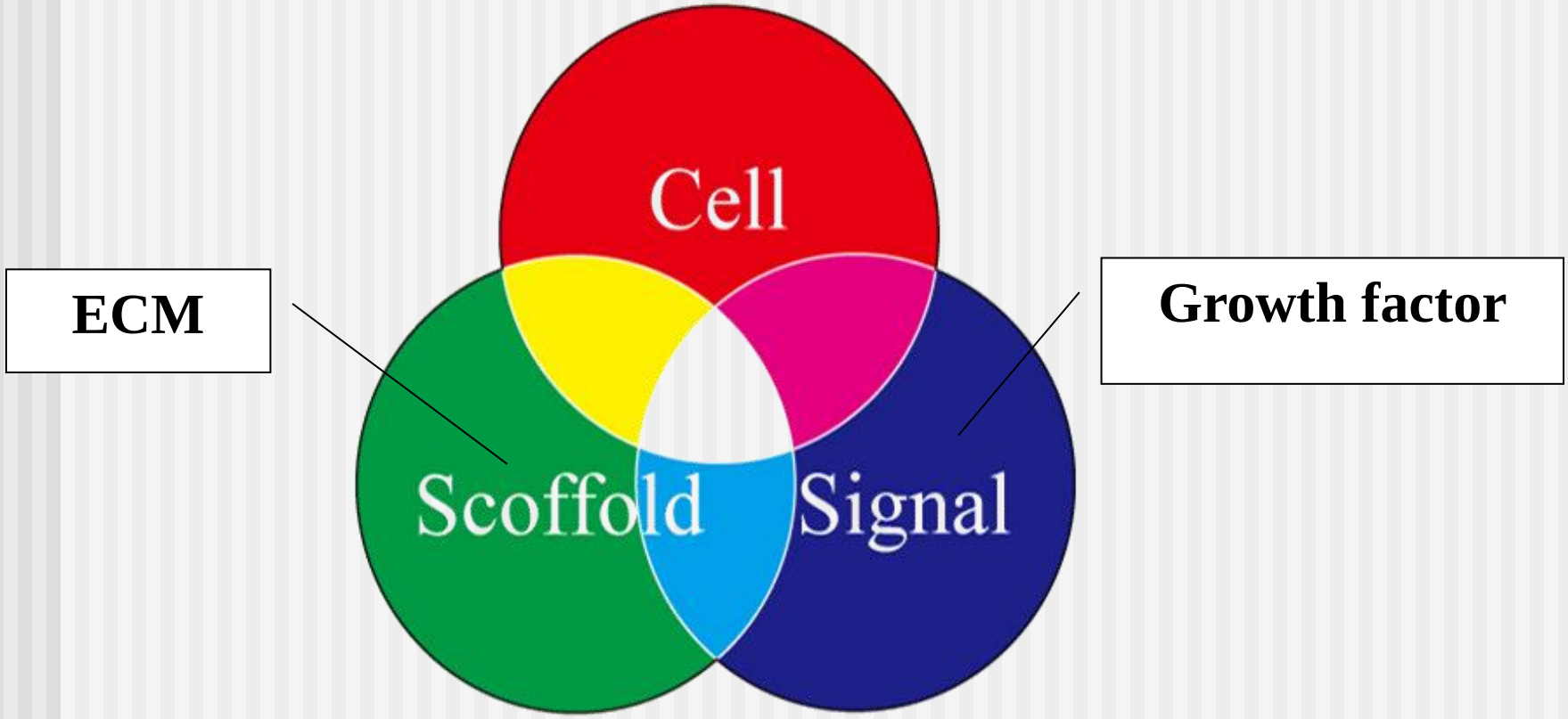
→ A tool box to facilitate good science

Practical conclusions for young(old) scientist

- A pre & postdoctoral training : interdisciplinery – question current hypotheses
- Add more strings to your bow : gain both from material and life science
- Seek an environment open to innovation: be critical about the track reward of your prospective workplace.

Regenerative Medicine

Effective and rapid restoration of functional tissue as a therapeutic response to injury, disease, or aging.



Traditional Biomaterials

Materials	Replaced tissues
Polymer – nylon, daklon, teflon	Suture, artificial artery, Vein, nose, ear, tooth
Metal - titanium	Bone plate
Potery	tooth
composite	Artificial valve

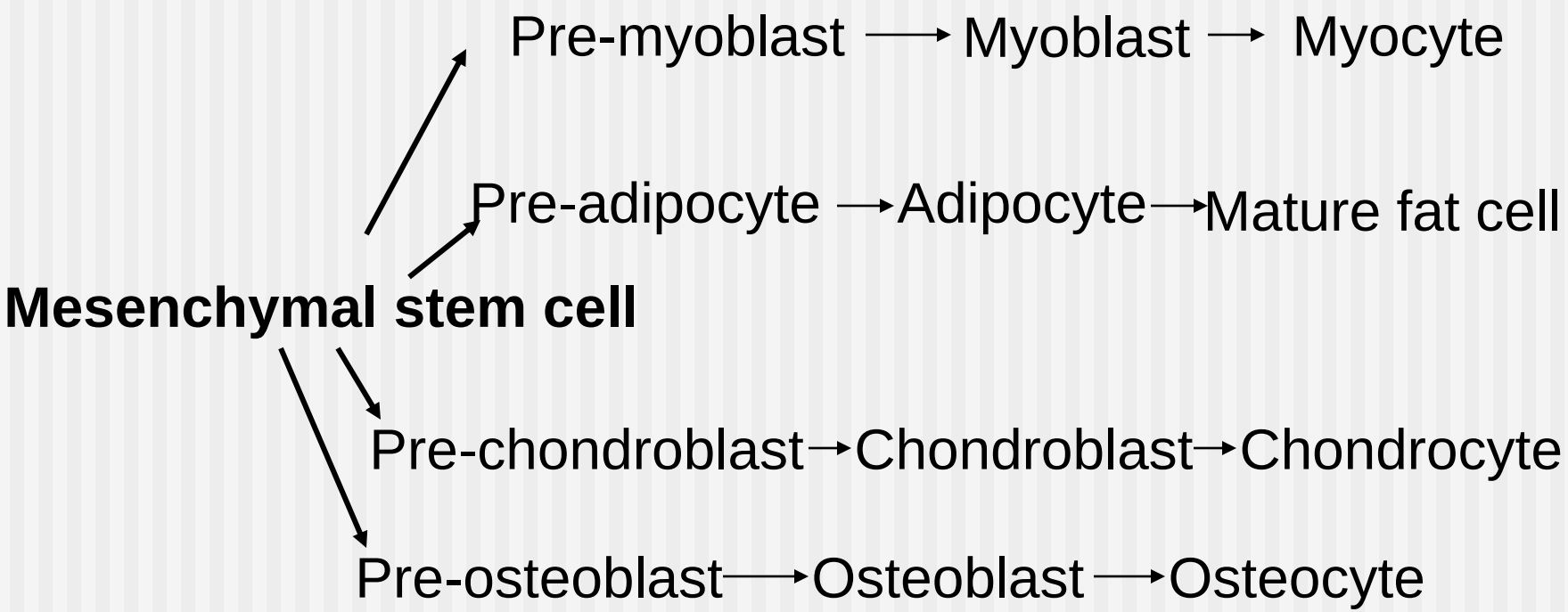
Features of Modern Biomedical Materials

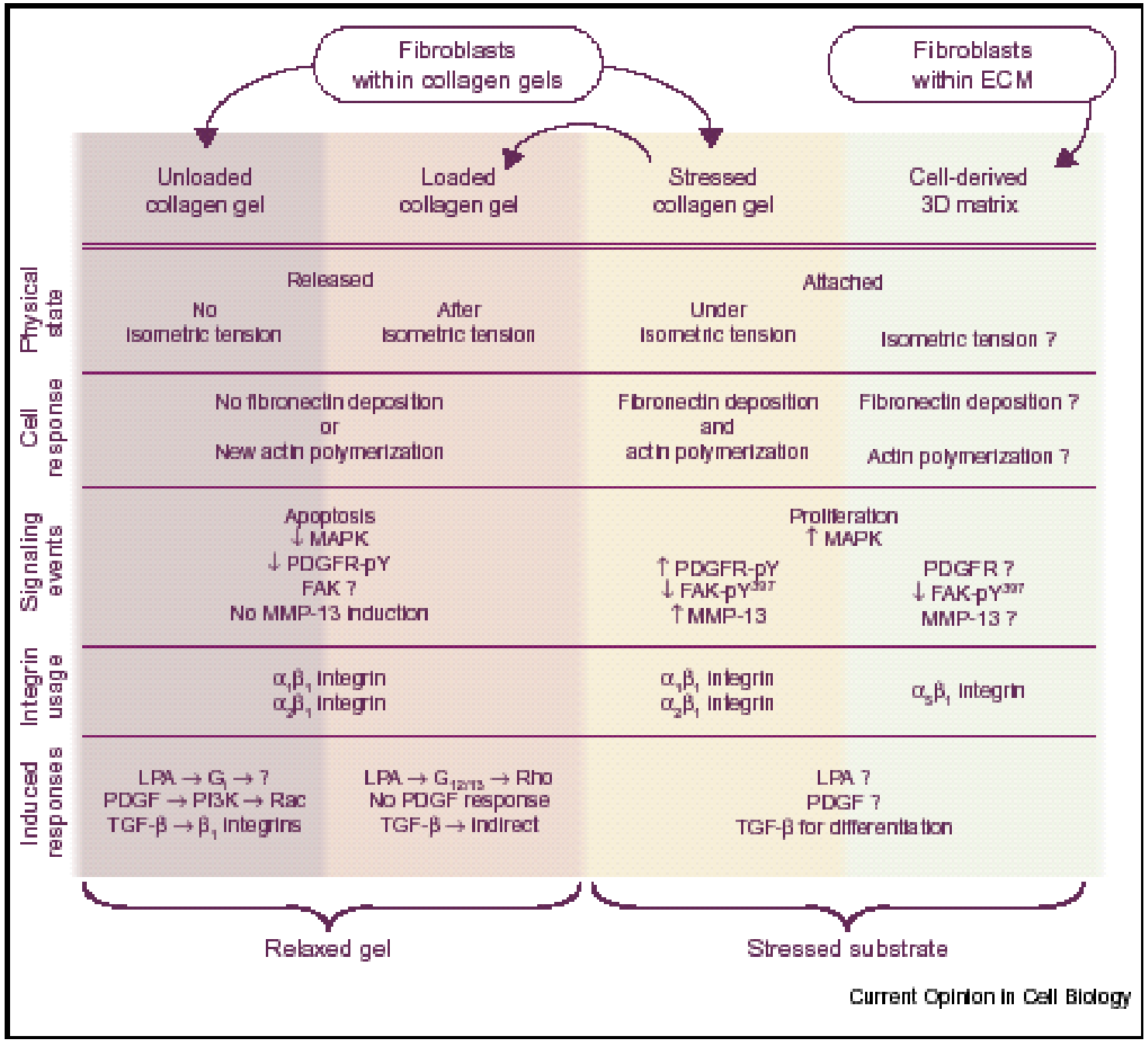
Biocompatible

Biodegradable/Bioadsorbable

Bioactive

Stem Cell Differentiation





Current Opinion in Cell Biology 2002, **14**:633–639

Microenvironment = “Niche”

Niches are local tissue microenvironments that maintain and regulate stem cells.

Mechanisms of stem cell maintenance are key to the regulation of homeostasis and likely contribute to aging and tumorigenesis when altered during adulthood.

Cell 132, 598–611, February 22, 2008

Identifying Niches

A niche consists of a local tissue microenvironment capable of
housing and maintaining one or more stem cells ……

a candidate niche should be transiently
depleted of its full complement of stem cells and then shown to take up and maintain a newly introduced stem cell.
This provides evidence that the niche microenvironment is localized
and not a general tissue property.

Cell 132, 598–611, February 22, 2008

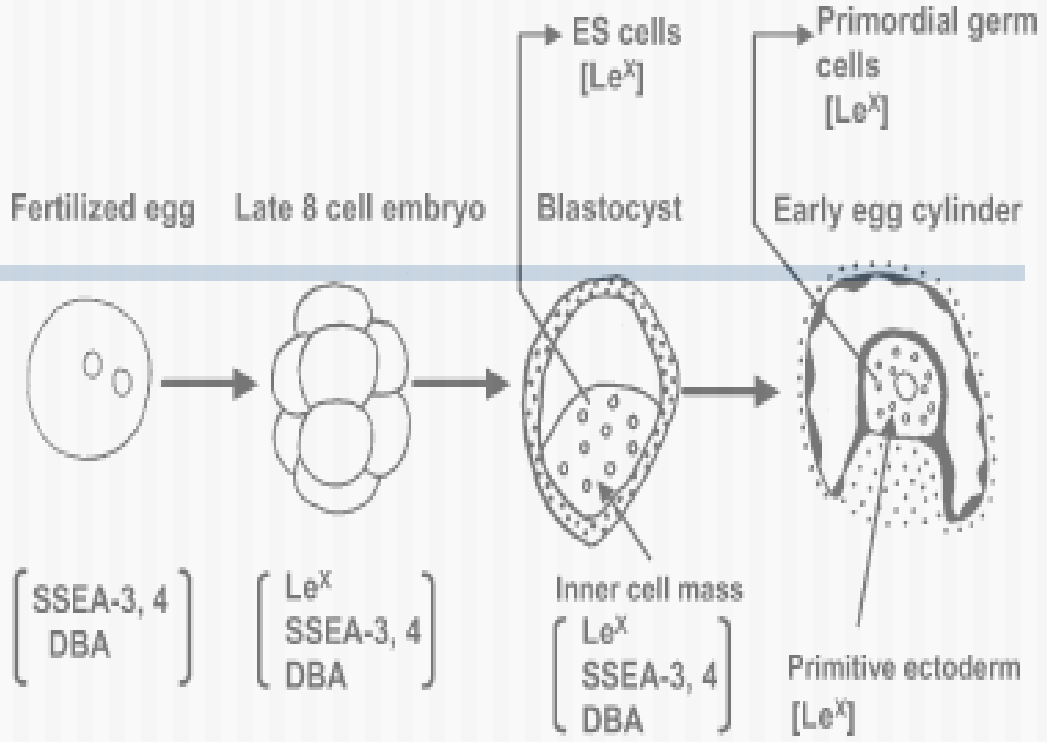


Figure 1. Expression of carbohydrate markers in early embryos and stem cells in mice. Expressed markers are written in parenthesis. More detailed profile of their expression is described in a review [2]: in the inner cell mass, Le^X expression changes from ± to + and DBA expression changes from + to ± during blastocyst development. Le^X(SSEA-1, 4C9, LTA): Galβ1-4(Fucα1-3)GlcNAc, SSEA-3: R-3Gal NAcβ1-3Galα1-4R′, SSEA-4: NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4R, DBA(Sd^a antigen): NeuAcα2-3(GalNAcβ1-4)Galβ1-4GlcNAc.

Table 1. Expression of Le^X in stem cells and early embryonic cells of mice and human

Cells	*Mice*	*Human*
ES cells	+	−
EC cells	+	−
EG cells	+	+
Neural stem cells	+	+
Hematopoietic stem cells	−	−
Inner cell mass cells	±+	−
Primitive ectoderm cells	+	?
Primordial germ cells	+	?

+, expression; −, no expression; ±+ , weak expression ; ?, unknown

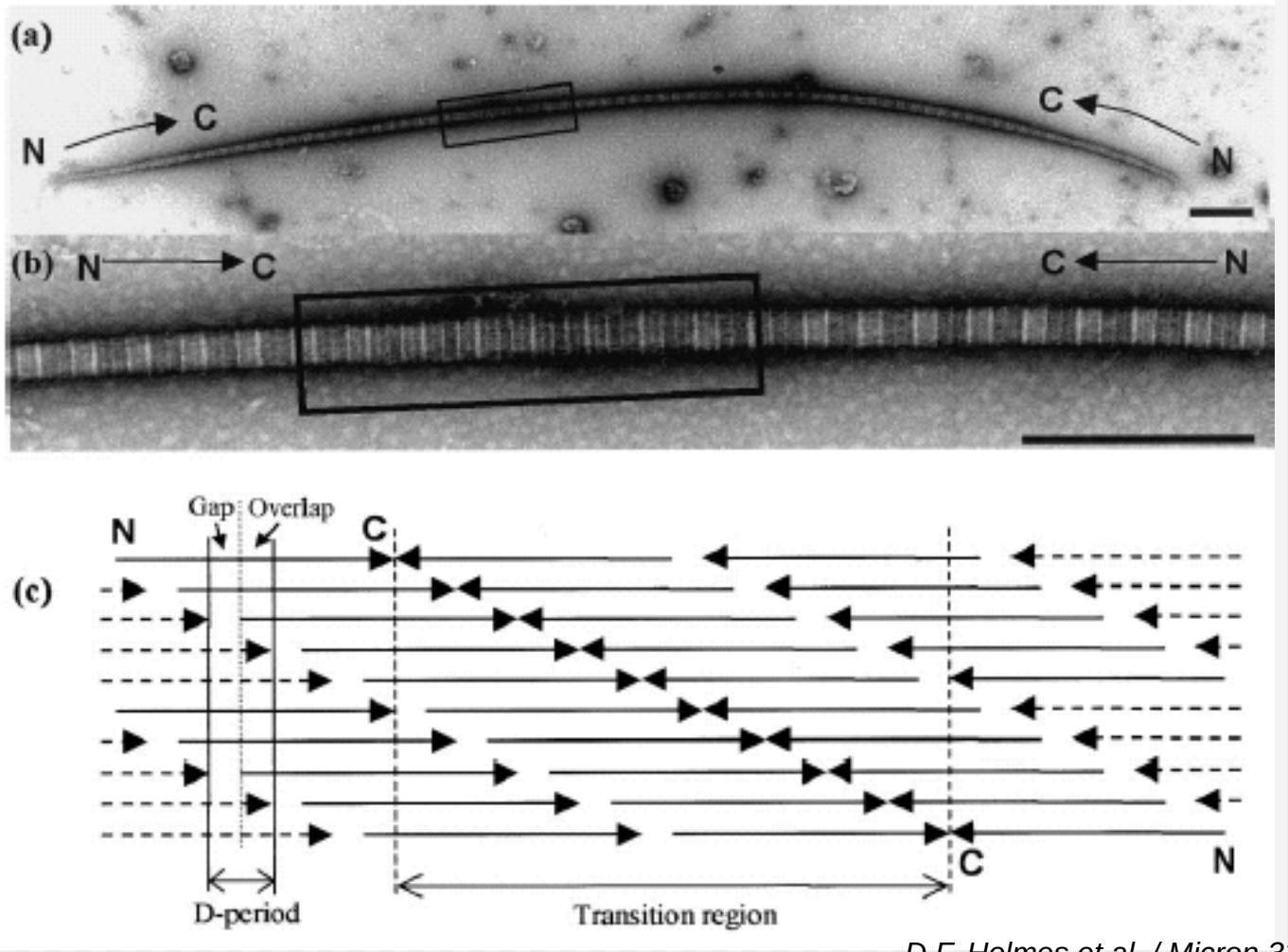
Cell, 2007	Science, 2007
Oct3/4	Oct3/4
Sox2	Sox2
Klf4	Lin28
C-myc	NanoG

Fibroblast → Stem Cell

Niches in Disease:

- Cancer Stem Cell Niches
- Niche Aging

SEM of collagen



D.F. Holmes et al. / Micron 32 (2001) 273–285

Collagen fiber

Gly-X-Y, X, Y, proline or hydroxyproline

Triple helix (enzyme digestion, cross linked by deamination on specific lysine and hydroxylysine)

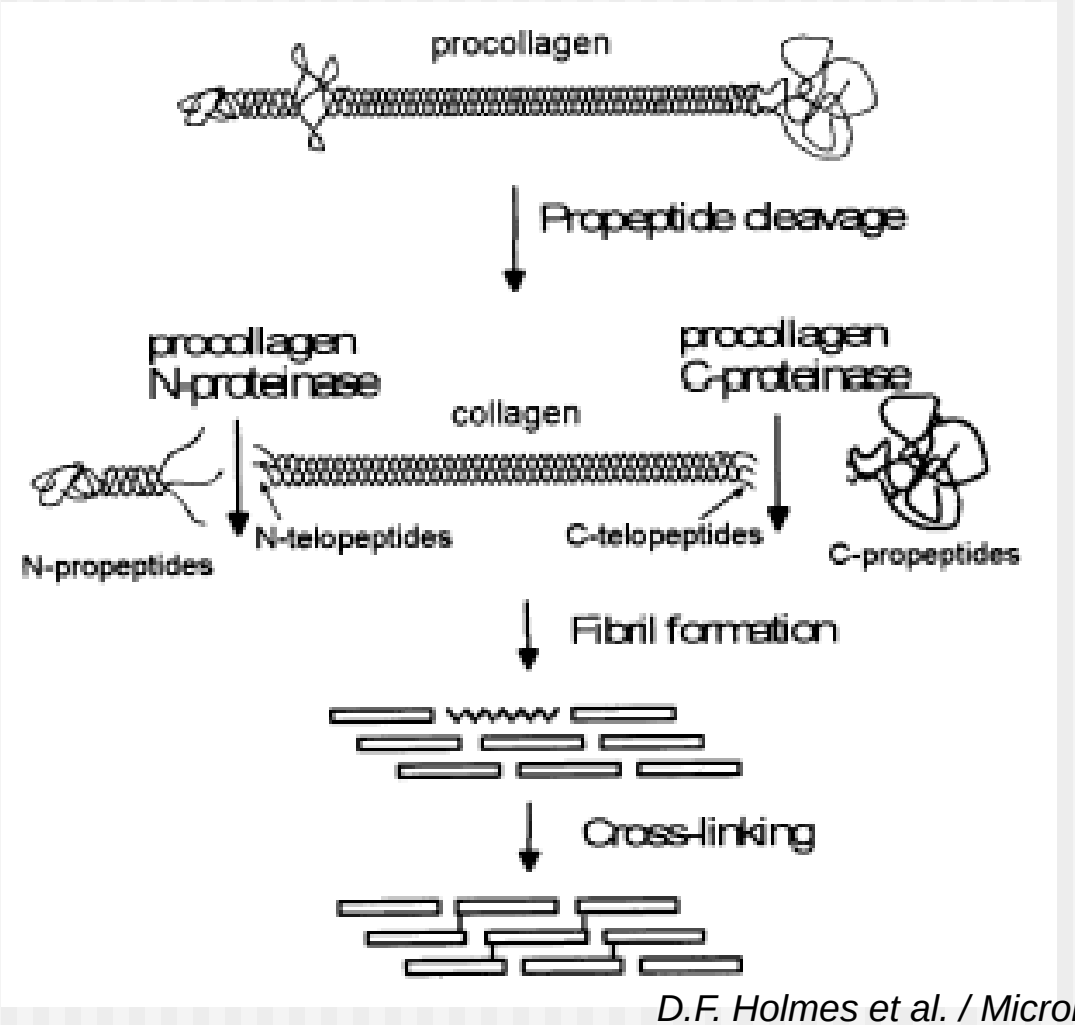
67 nm axial periodicity in most tissues
(65 nm in vertebrate skin)

Fubril diameters depend on tissue type and
stage of development
(20-500 nm for vertebrate tissue)

Entire fibrils with lengths in the range 1-100 um

Collagen Assembly

Gly-X-Y



D.F. Holmes et al. / Micron 32 (2001) 273–285

Size of collagen

Table 1
Summary of fibril growth characteristics measured on isolated fibrils from different assembly systems. The first two columns are for fibrils formed in vitro from purified components; the second two columns are for fibrils formed in vivo

Fibril growth characteristics	Acid-extracted collagen (early fibrils)[a]	pCcollagen + C-proteinase ($E : S = 50 : 50$)[b]	Chick tendon (12d embryo)[c]	Sea cucumber dermis[d]
Length range (μm)	2–16[e]	55–240	1–46	14–444
Maximum M/L (kDa/nm)	150	46000	535	16000
Maximum diameter[f] (nm)	21	364	37	208
Mean mass slope of tips (molecules/D-period) (S.D. are shown in brackets)	N-tips: 4.8 (0.4) C-tips: 10.4 (0.6)	α-tips: 17 (1.4) β-tips: 113 (35)	24.9 (10)	6.0 (1.4) (Length <100μm)
Unipolar (UP) or N,N-bipolar (BP)	UP	BP	UP + BP	BP
Abrupt change in mass slope of tips	No	No	Yes	Yes
Diameter limitation	Yes	$M/L \propto L$	Yes	$(D_{\text{eff}} - 14) \propto L$
Inter-fibrillar fusion	Yes	No	Yes	No

[a] Holmes (1978); Holmes and Chapman (1979).
[b] Holmes et al. (1992).
[c] Holmes et al. (1998); Graham et al. (2000).
[d] Trotter et al. (1998).
[e] Fibrils up to180 μm in length have been grown by seeding collagen solutions with reconstituted early fibrils (Haworth, 1972).
[f] Effective diameter values derived from M/L values.

D.F. Holmes et al. / Micron 32 (2001) 273–285

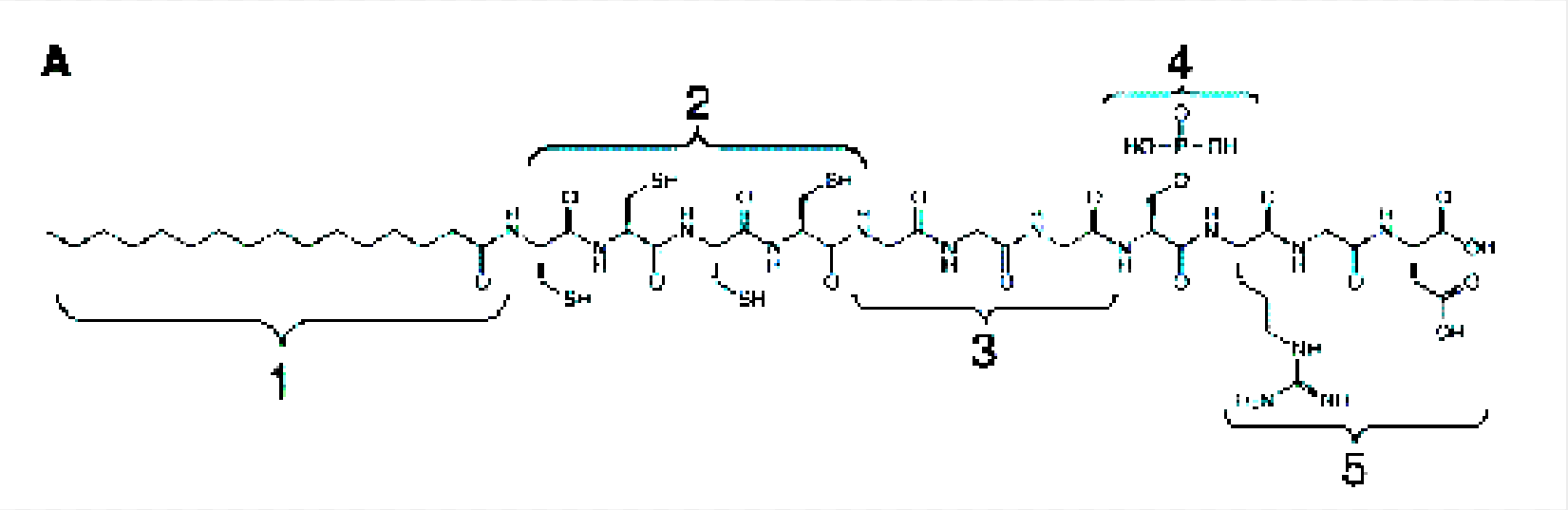
Application of Nanotechnology on Biomaterials

Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers

294, 2001 Science

The peptide amphiphile

294, 2001 Science



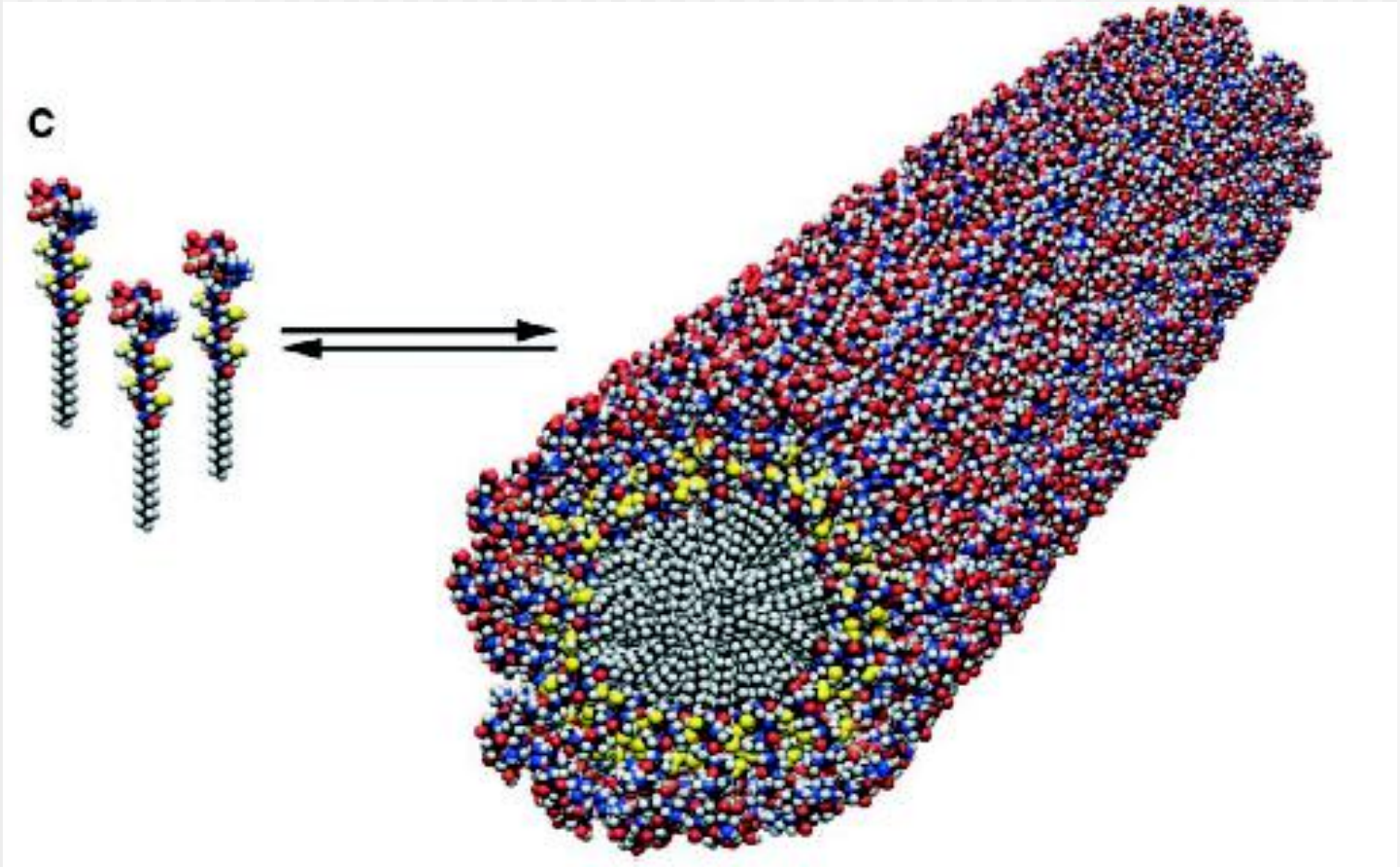
1. Alkyl tail (hydrophobic)
2. Four Cys
3. Three Gly (flexibility)
4. Phosphorylated Ser (interact with Ca and hydroxyapatite
5. R-G-D (cell adhesion)

Molecular Model of peptide amphiphile

294, 2001 Science

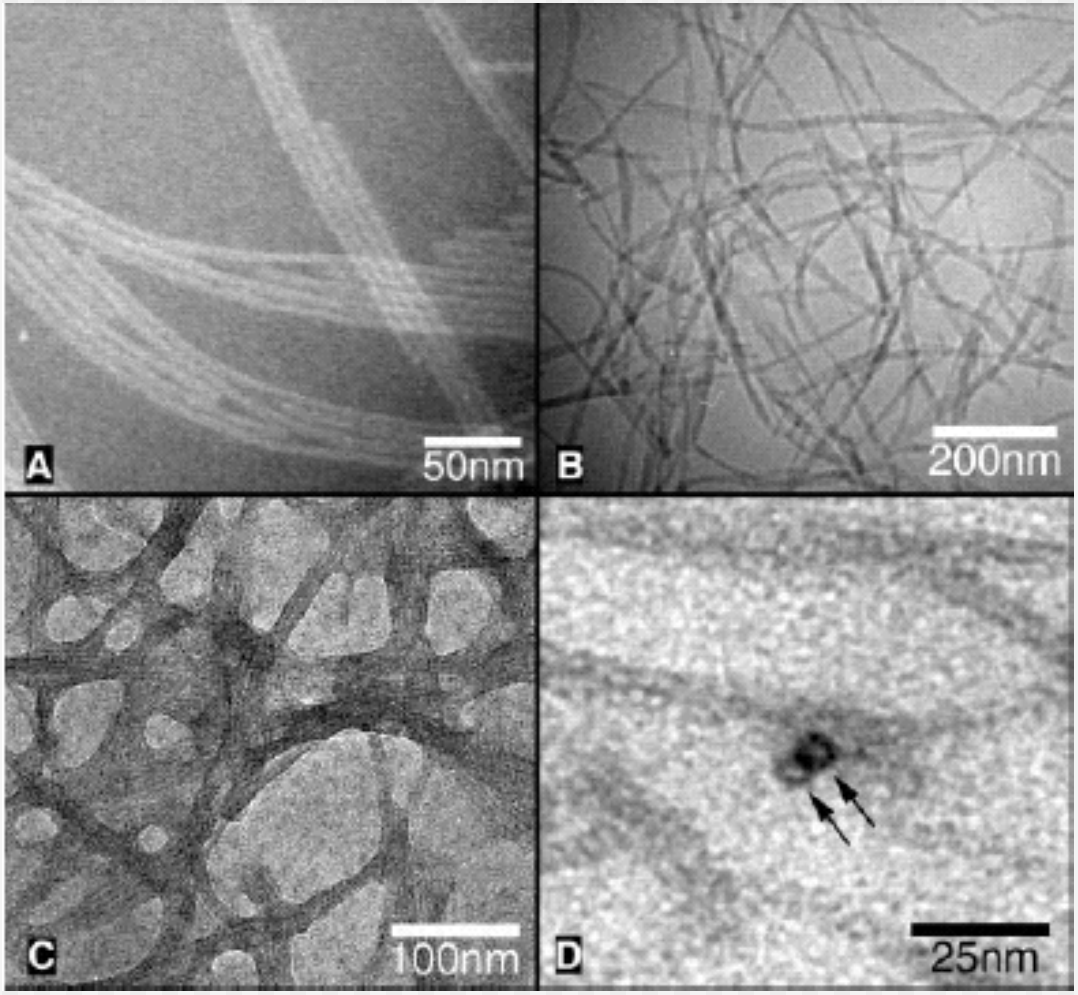
Self-assembly of peptide amphiphile

294, 2001 Science



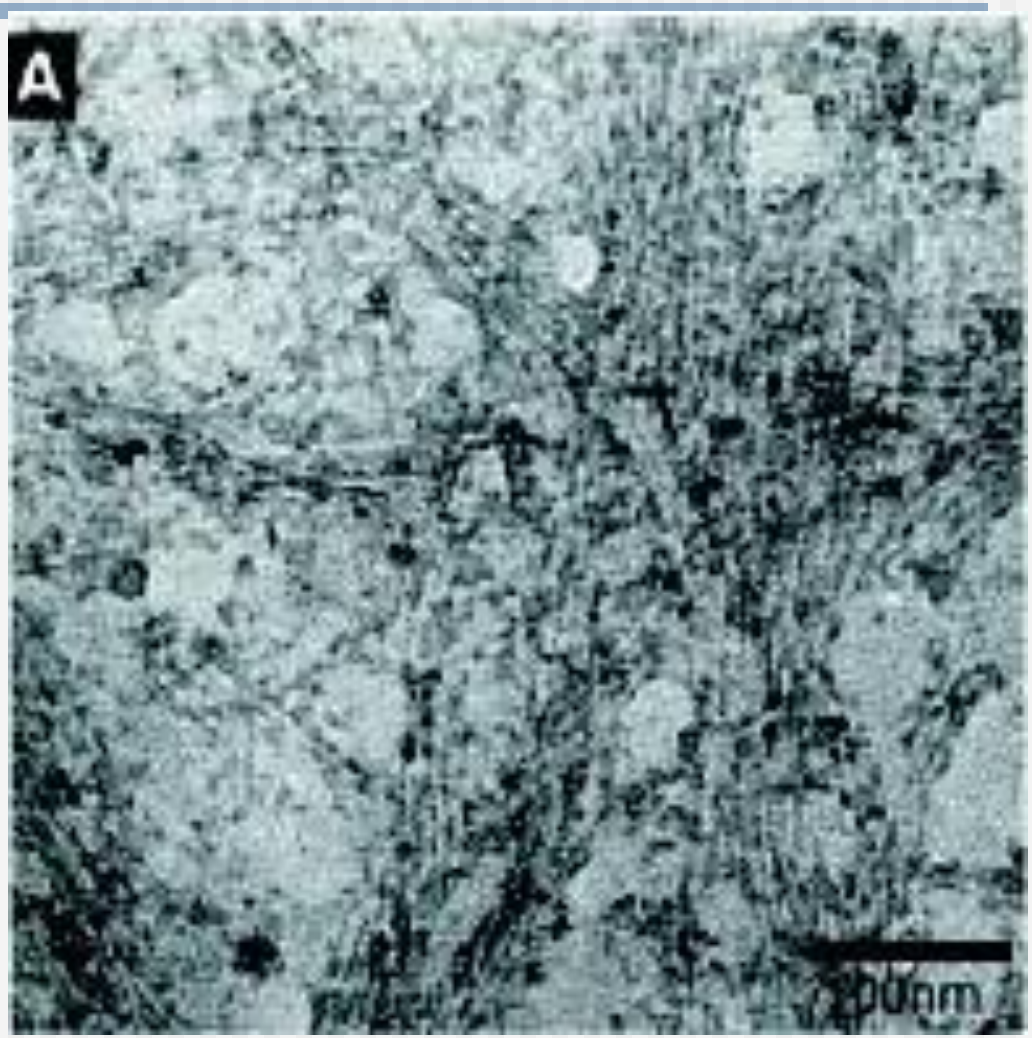
TEM of nanofiber self-assembly

294, 2001 Science



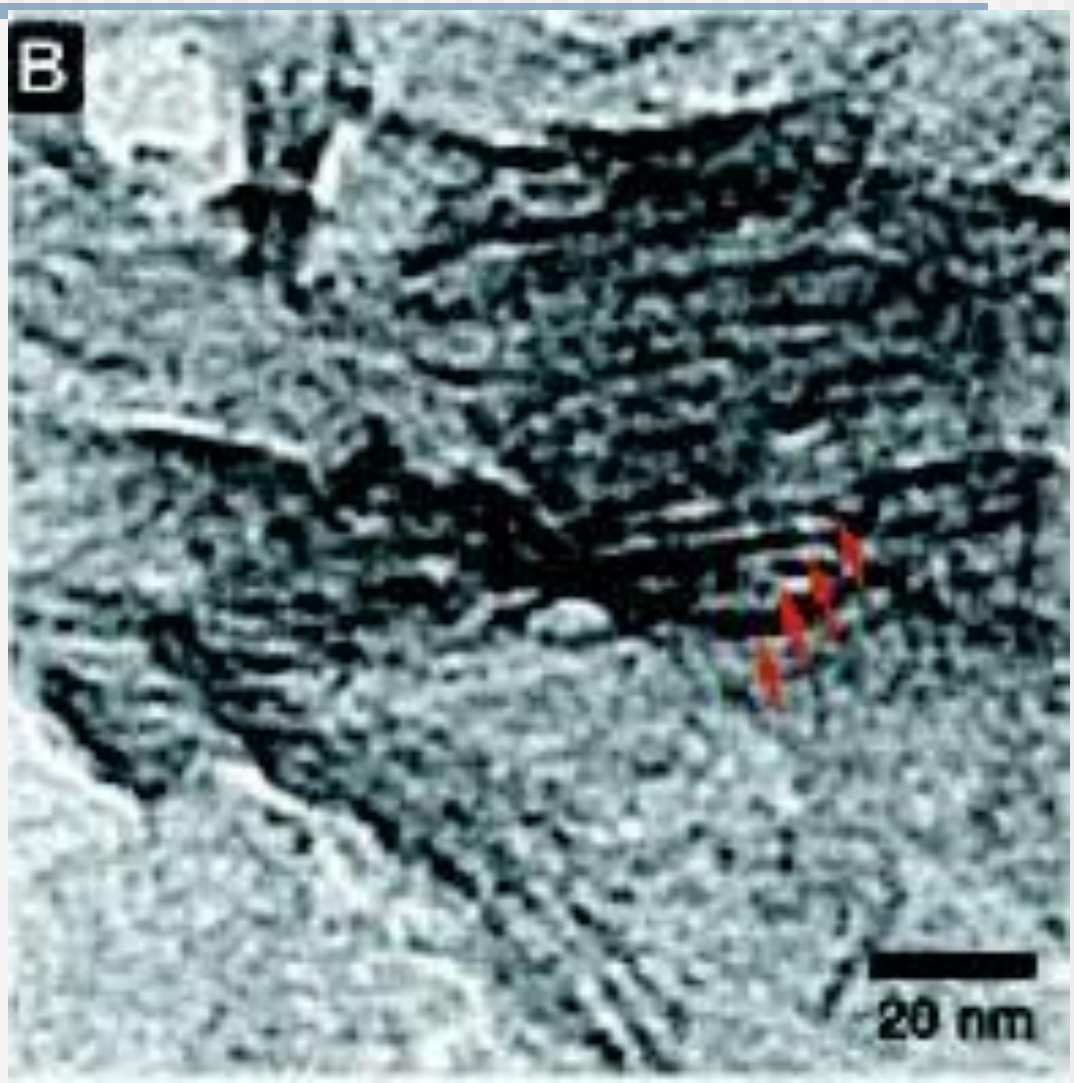
Cross-linked of PA

294, 2001 Science



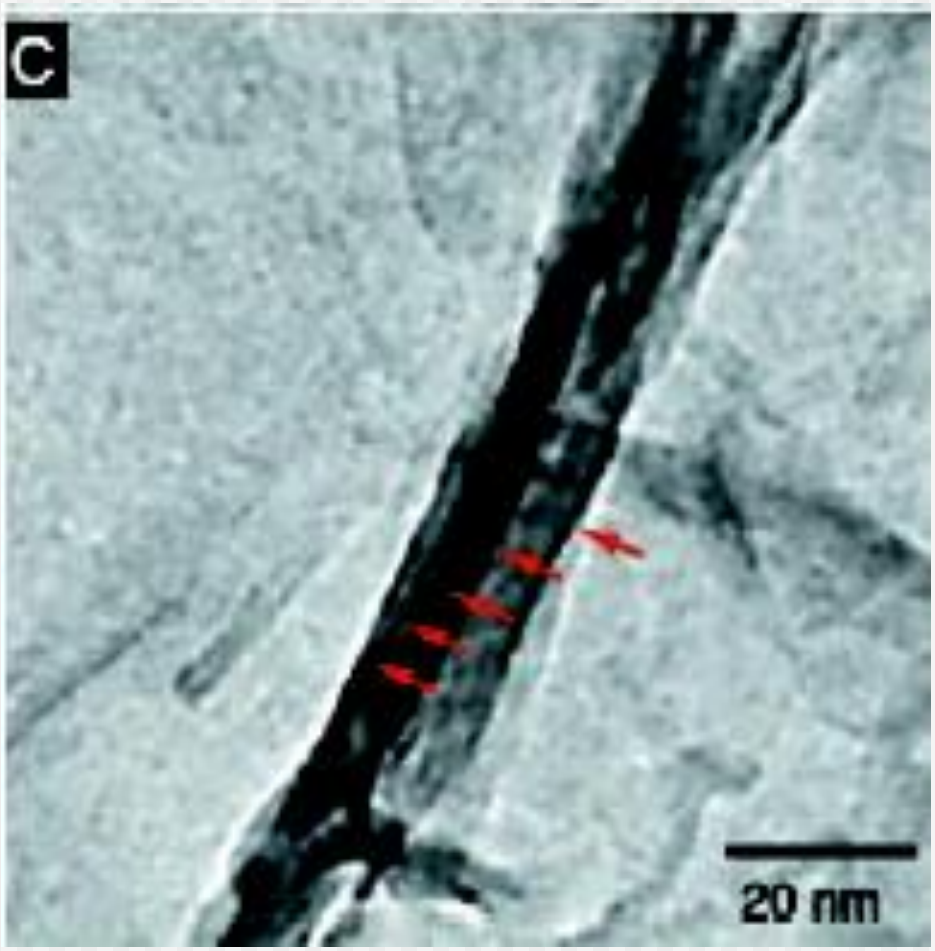
HA crystal forming

294, 2001 Science



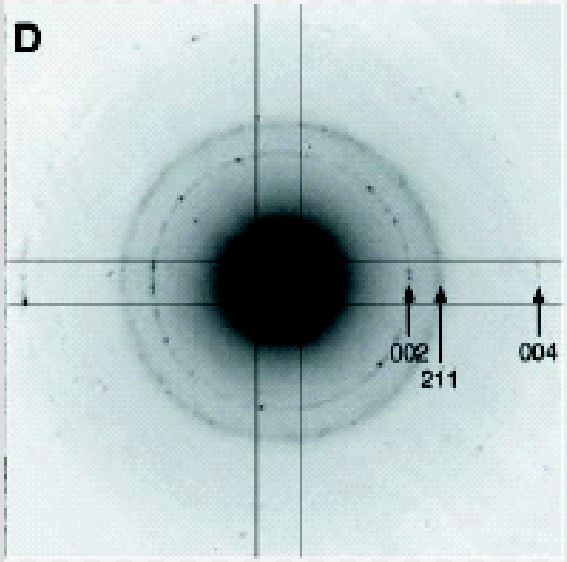
Mature HA crystal formation

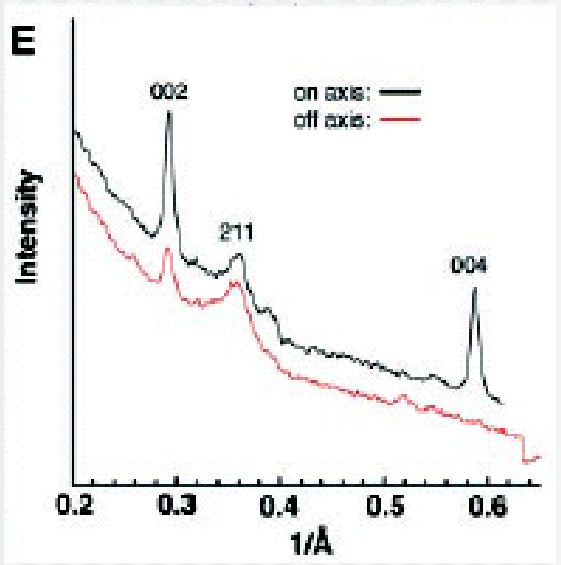
294, 2001 Science

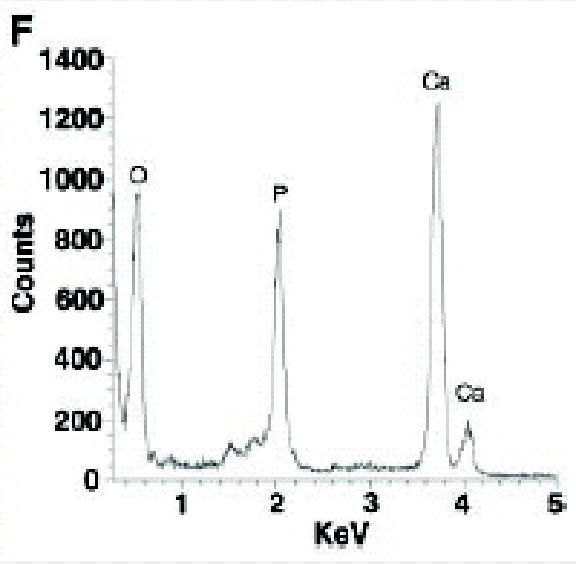


Characteristics of PA assembly

294, 2001 Science







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Hybrid bone implants: Self-assembly of peptide amphiphile nanofibers within porous titanium

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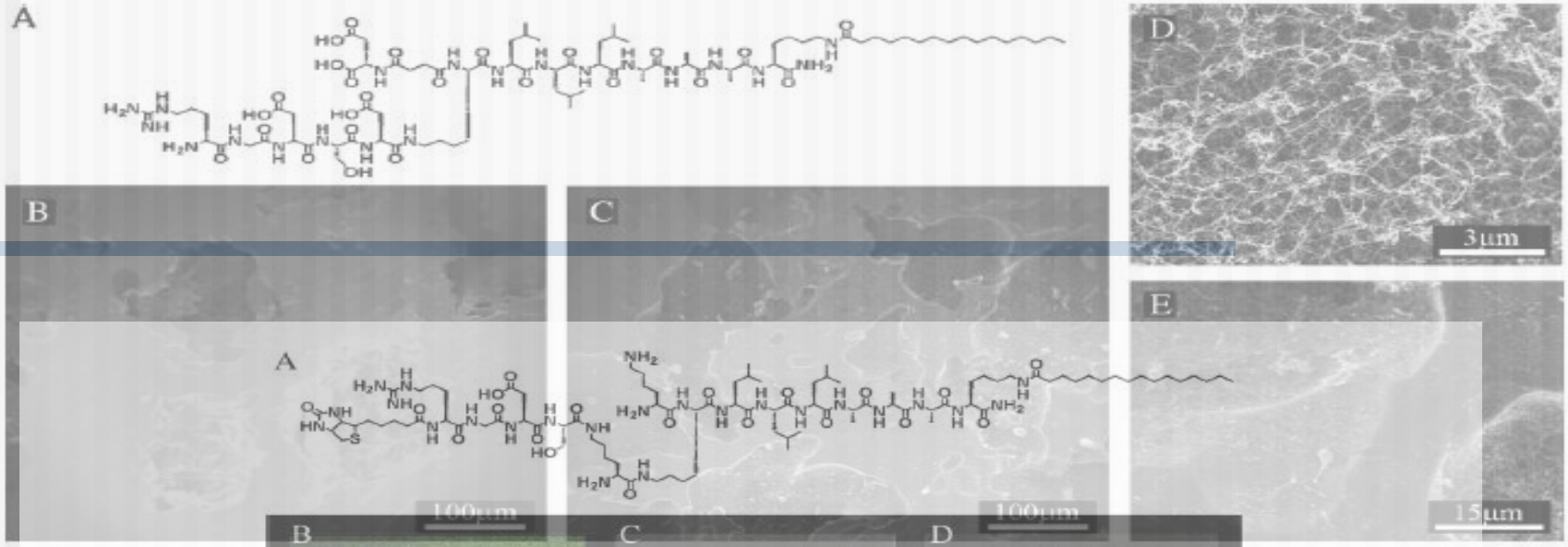

Fig. 2. (A) Chemical structure of the peptide amphiphile (PA) used to infiltrate and fill the pores of the Ti–6Al–4V foam. Scanning electron microscopy (SEM) images of (B) the bare Ti–6Al–4V foam, (C) Ti–6Al–4V foam filled with PA gel, (D) higher magnification of the self-assembled PA nanofibers forming a three-dimensional matrix within the pores, and (E) higher magnification of the PA coating the Ti–6Al–4V foam surface and filling the pores.

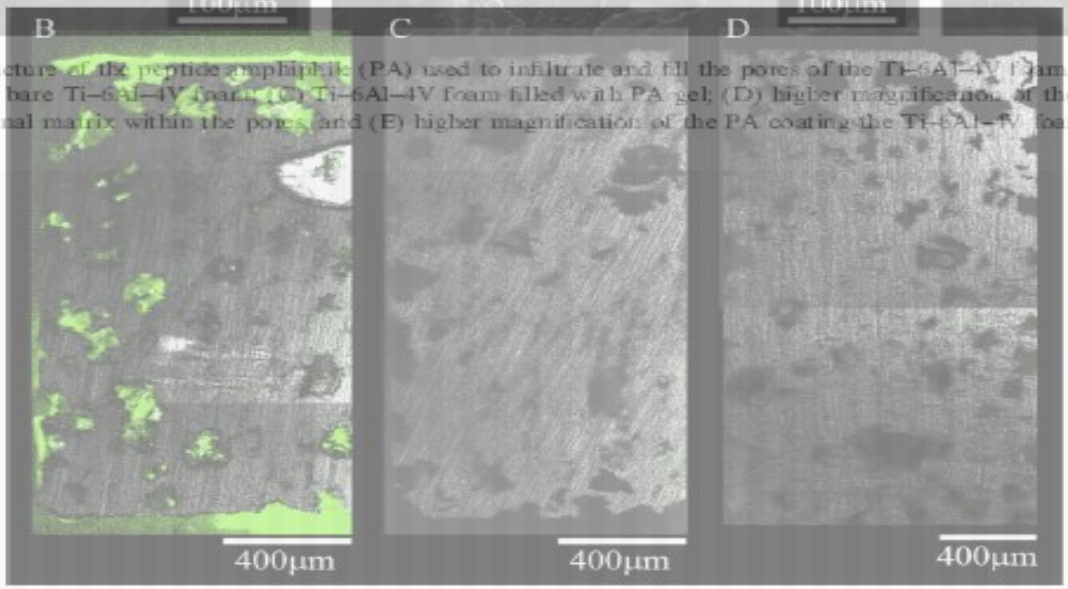

Fig. 3. Chemical structure of the biotinylated PA used for the confocal microscopy without cells (A), and the resulting confocal microscopy images of the biotinylated PA–Ti hybrids embedded in acrylic and cross-sectioned, showing (B) the fluorescence of avidin–FITC bound to biotinylated PA gelled throughout the cross-section of Ti–6Al–4V foam, (C) no fluorescence from a control sample of Ti–6Al–4V foam and biotin–PA without avidin–FITC, and (D) no fluorescence from a second control of Ti–6Al–4V in acrylic without PA.

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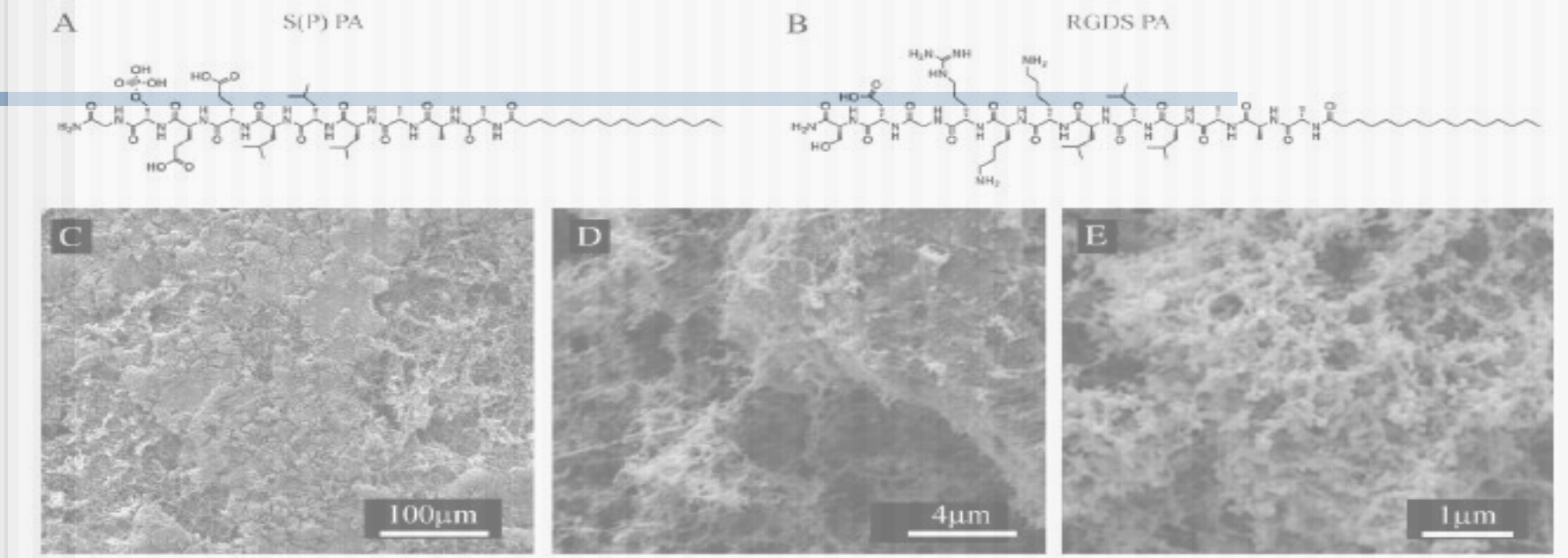

Fig. 4. Chemical structures of the S(P) PA and RGDS PA used to create PA–Ti hybrids for mineralization (A, B). SEM images (C–E) show nanoscale bead-like mineral formation on the PA nanofibers. High magnification images (D–E) show the mineral formation only on the nanofibers, and not on the metal surface, indicating templation on the PA nanofibers. EDS quantification reveals a Ca:P ratio for the mineral as 1.71 ± 0.18, in line with hydroxyapatite.

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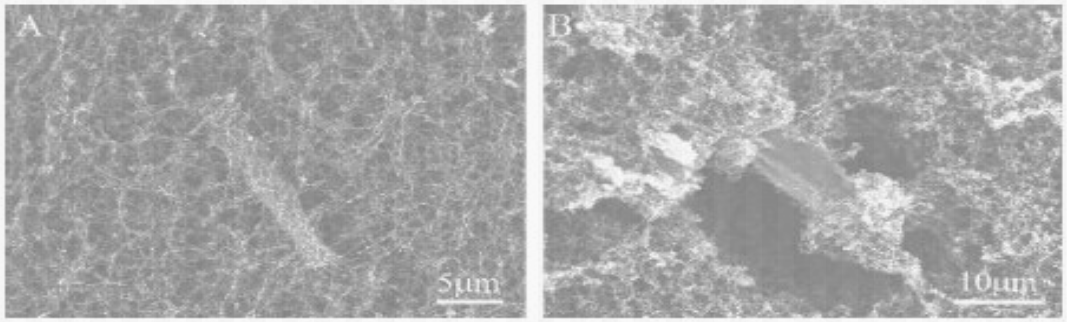

Fig. 6. SEM of GFP-transfected MC3T3-E1 cells encapsulated within PA–Ti hybrids. Cells encapsulated near the surface of the PA gel can be visualized spreading and pulling on the nanofibers presenting the RGDS cellular adhesion motif.

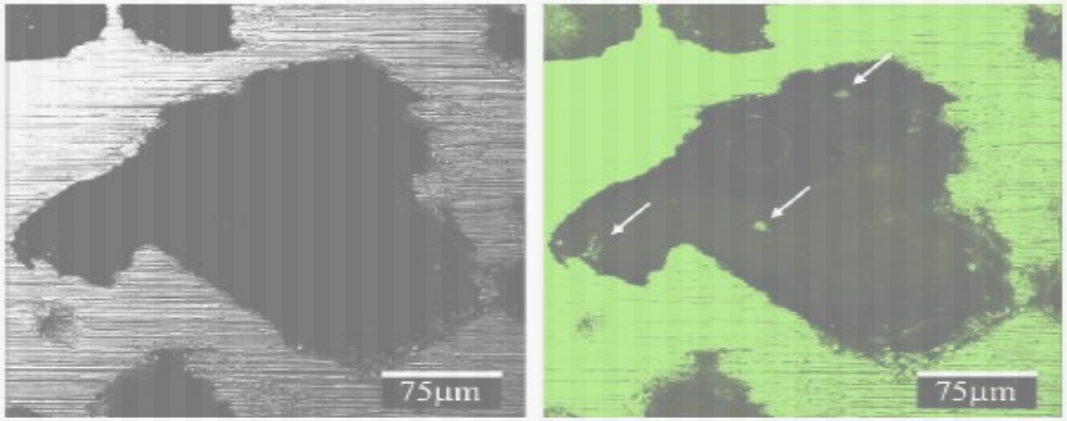

Fig. 7. Confocal microscopy of GFP-transfected MC3T3-E1 cells encapsulated within PA–Ti hybrids (cross-section). Left image is reflectance mode, while right image is fluorescence, showing cell suspended in an interior pore. The metal shows artificial fluorescence due to reflectance at the wavelength collected. The difference between the images is the true fluorescence of the cells, indicated by the arrows.

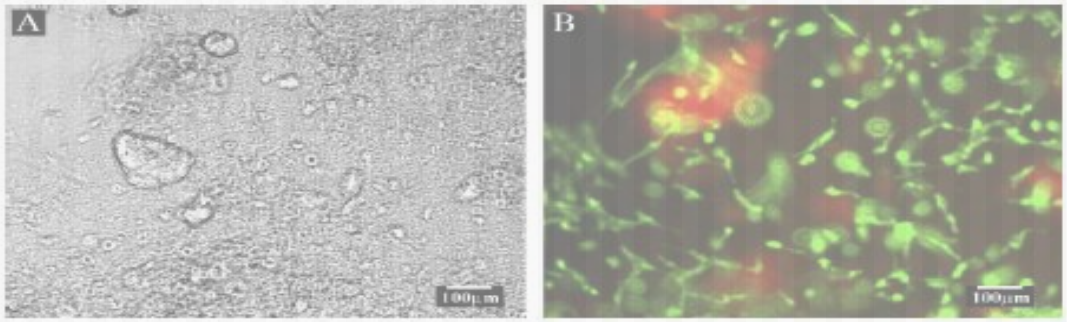

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Fig. 8. Optical (A) and fluorescence (B) microscopy images of non-transfected MC3T3-E1 cells encapsulated within the nanofiber matrix of the PA shown in Fig. 2A. Almost all cells fluoresced green due to the conversion of calcein AM to calcein, indicative of live cells. The hazy red areas are background fluorescence due to the interaction of the PA with EthD-1.

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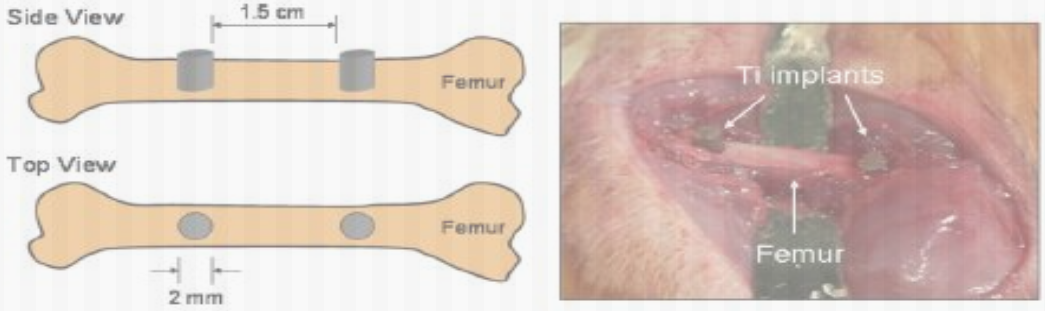

Fig. 9. Image depicts the rat femoral model used to assess the biocompatibility and osteoconductive/inductive potential of the Ti implants. Ti foam implants were positioned inside 2 mm diameter holes that were ~1.5 cm apart.

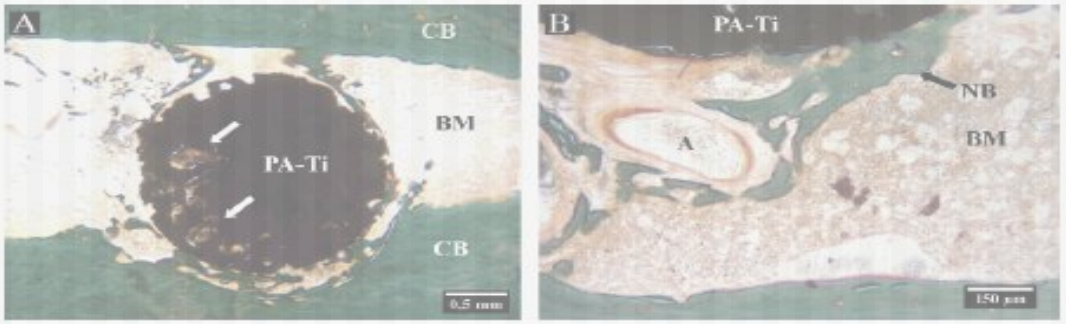

Fig. 10. Images of histological sections of a PA–Ti hybrid implanted in a rat femur after 4 weeks. Non-decalcified, plastic embedded samples were stained with Goldner's Trichrome. When staining bone, green indicates highly mineralized bone; red indicates newly formed, immature bone. As seen in Image A, new, mineralized bone is seen growing from the cortical bone (CM) towards the PA–Ti hybrid in the bone marrow (BM), and infiltrating the open porosity (arrows). Image B shows newly formed, fully mineralized bone adherent to the PA–Ti hybrid exterior. An artery (A) is observed adjacent to the implant, indicating neo-vascularization around the PA–Ti hybrid.

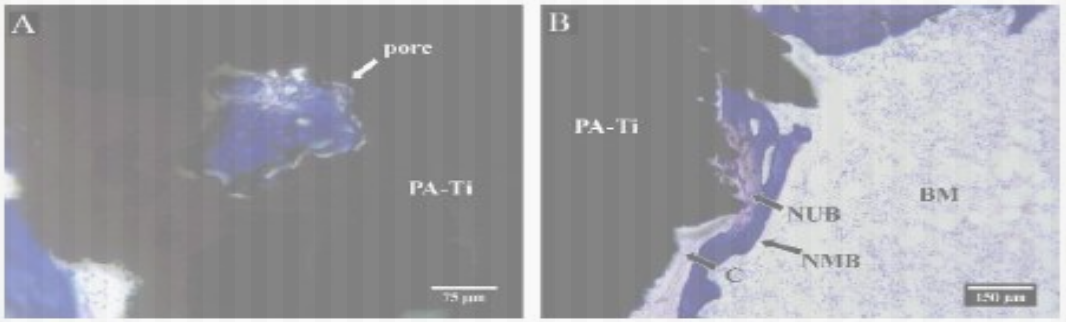

Fig. 11. Images of histological sections of a PA–Ti hybrid implanted in a rat femur after 4 weeks. Non-decalcified, plastic embedded samples were stained with methylene blue and basic fuchsin. Image A shows mineralized bone formation (blue) within an interior pore of the PA–Ti hybrid. Image B shows new bone formation adjacent to and into the PA–Ti hybrid. The location and formation of these bone spicules are evidence of osteoconduction, with new mineralized bone (NMB, deep blue) on the exterior, new unmineralized bone (NUB, pink) in the middle, and collagenous fibers (C) against the PA–Ti hybrid.

Cell Sheet Technology

Cell sheet engineering: a unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine

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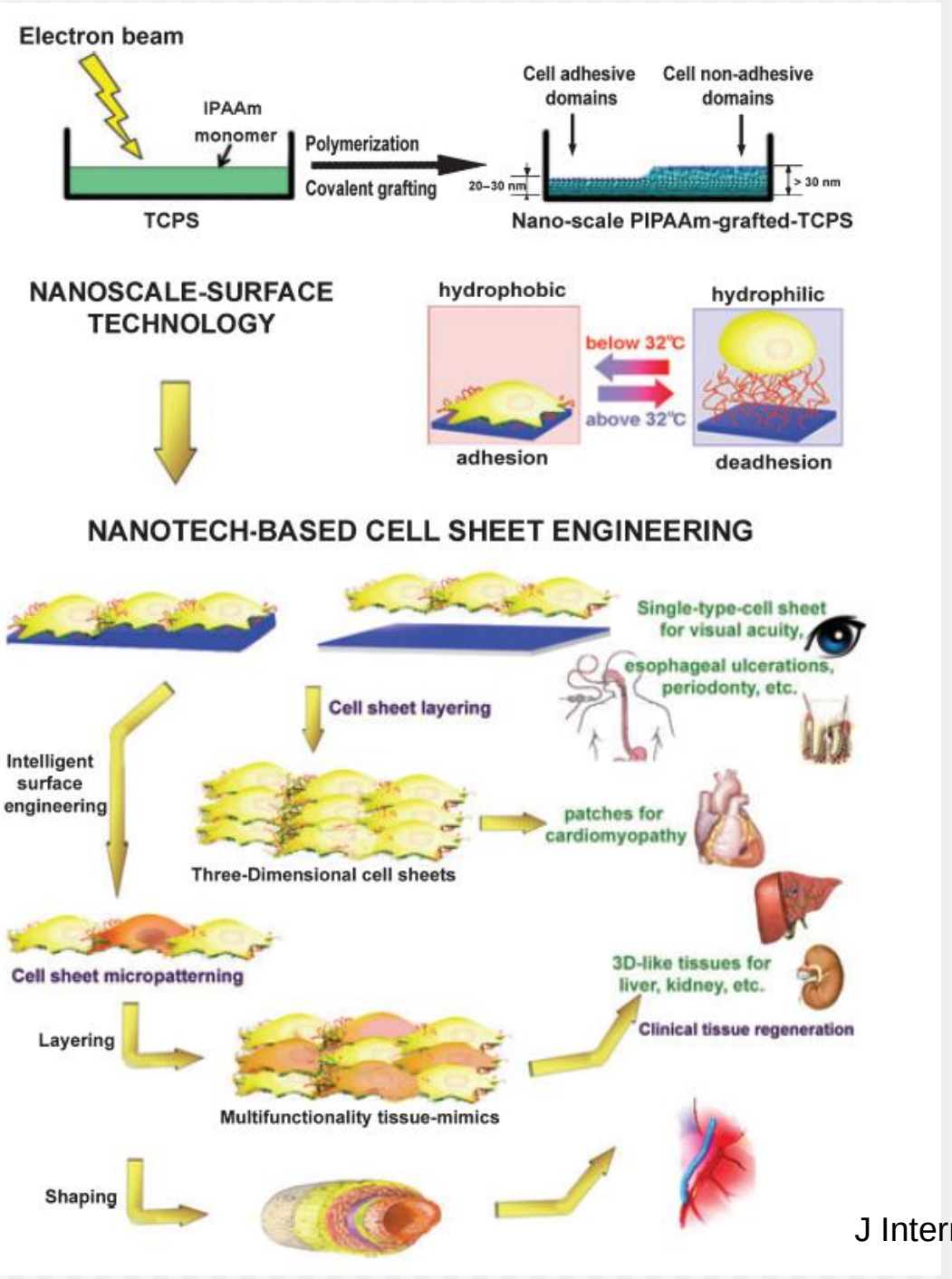
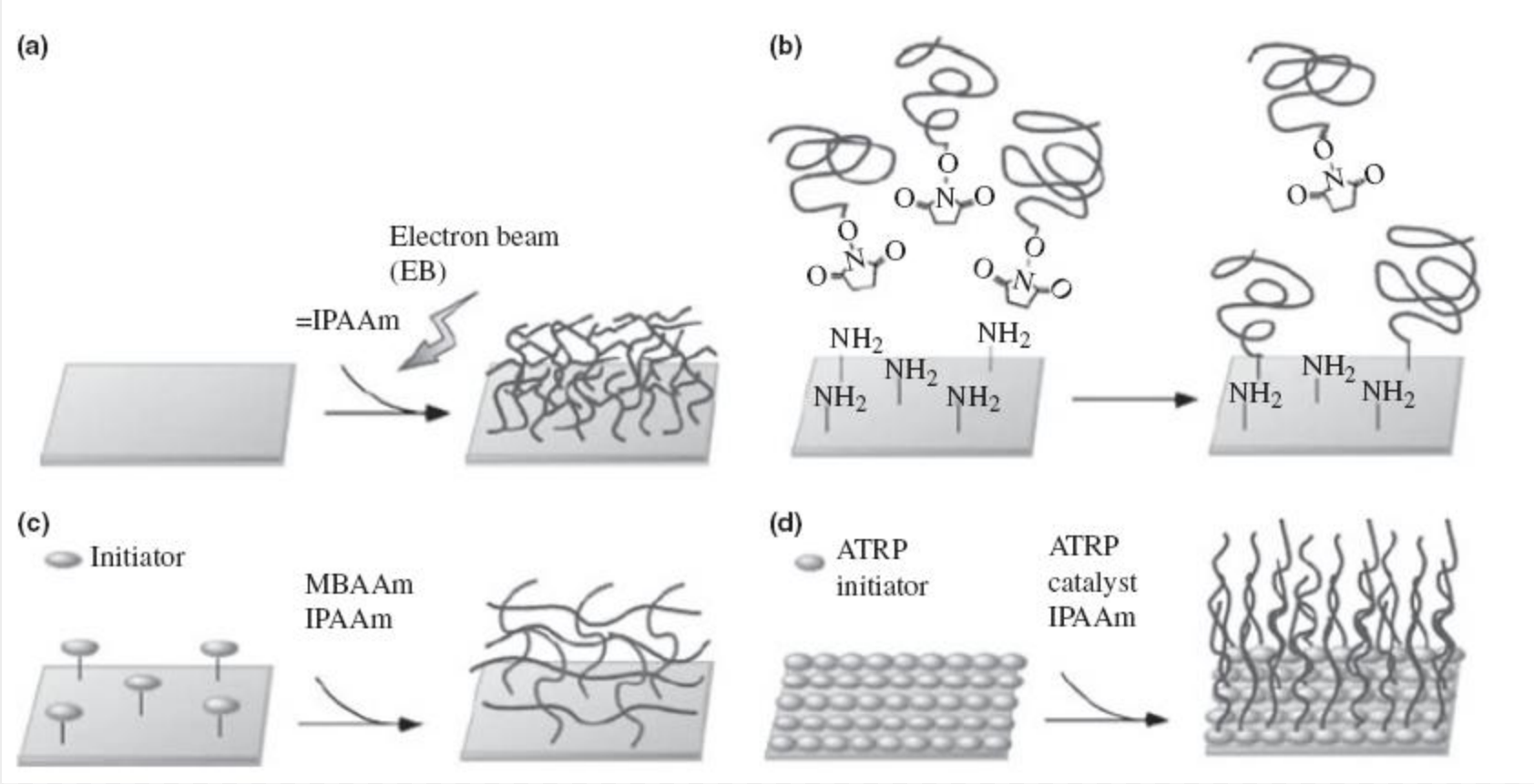
J Intern Med 2010; **267**: 54–70.



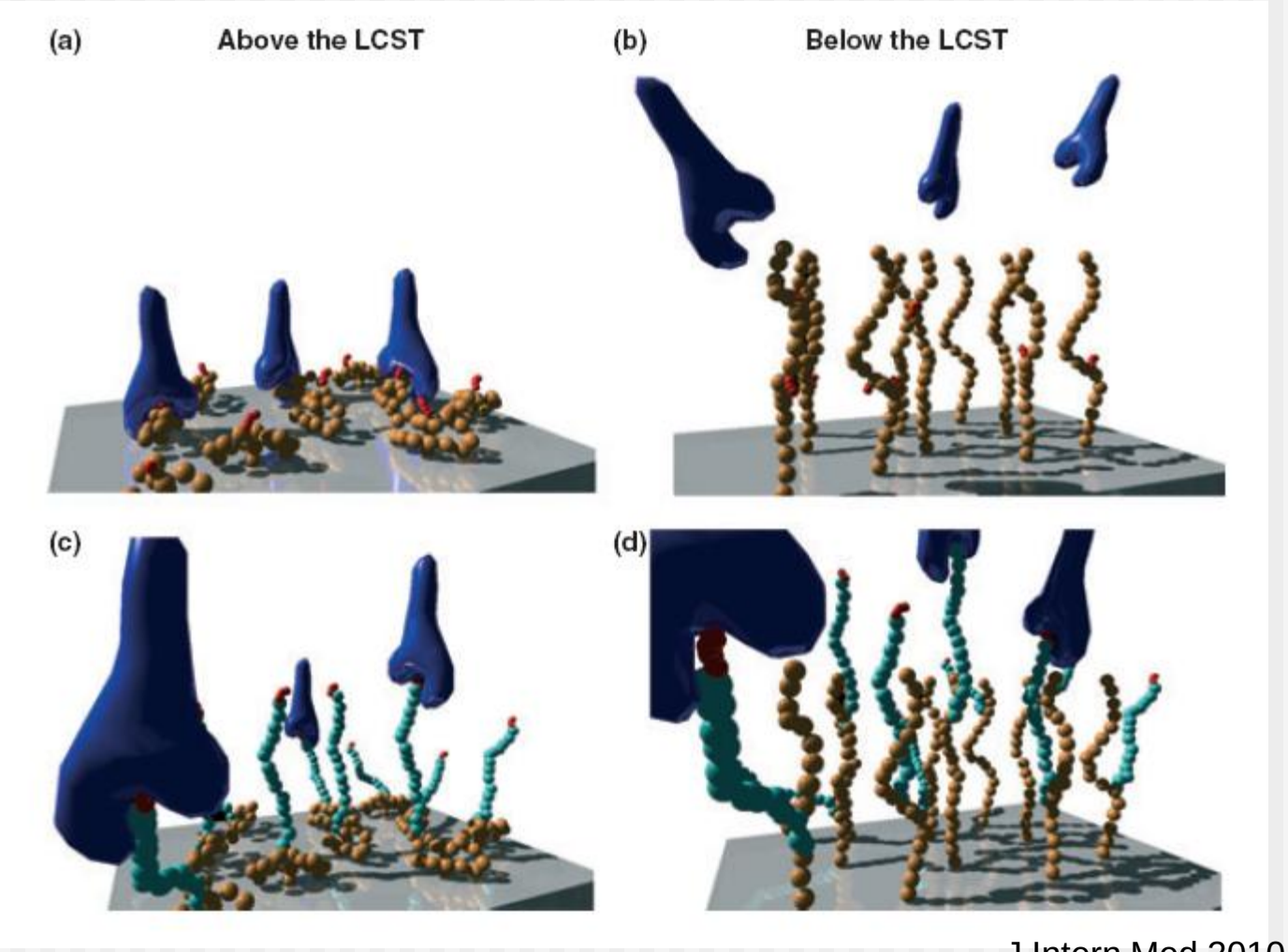
J Intern Med 2010, 267

Surface modification



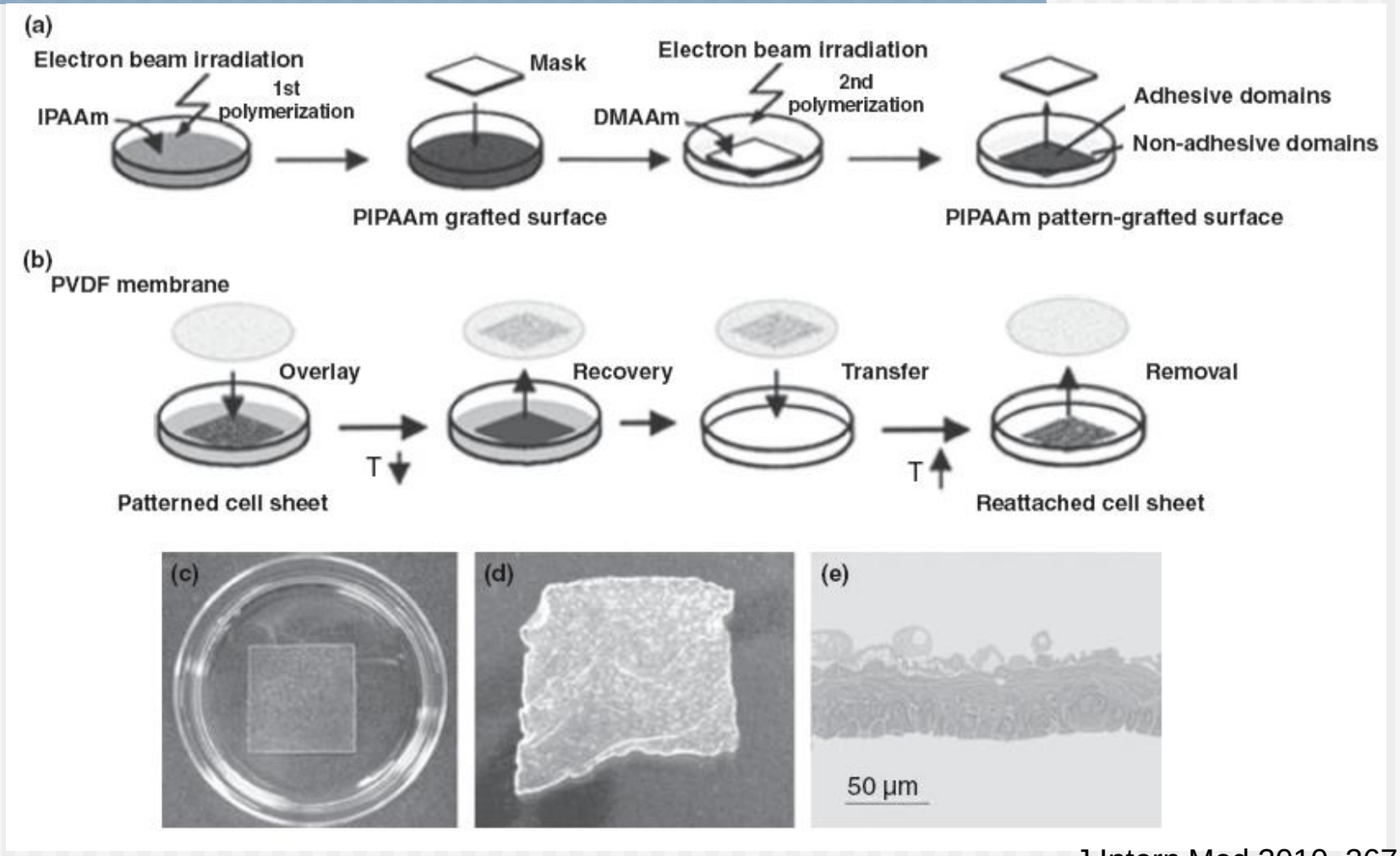
J Intern Med 2010, 267

Thermal-induced on-ff control of integrin-peptide binding



J Intern Med 2010, 267

Cell sheet harvest and transfer



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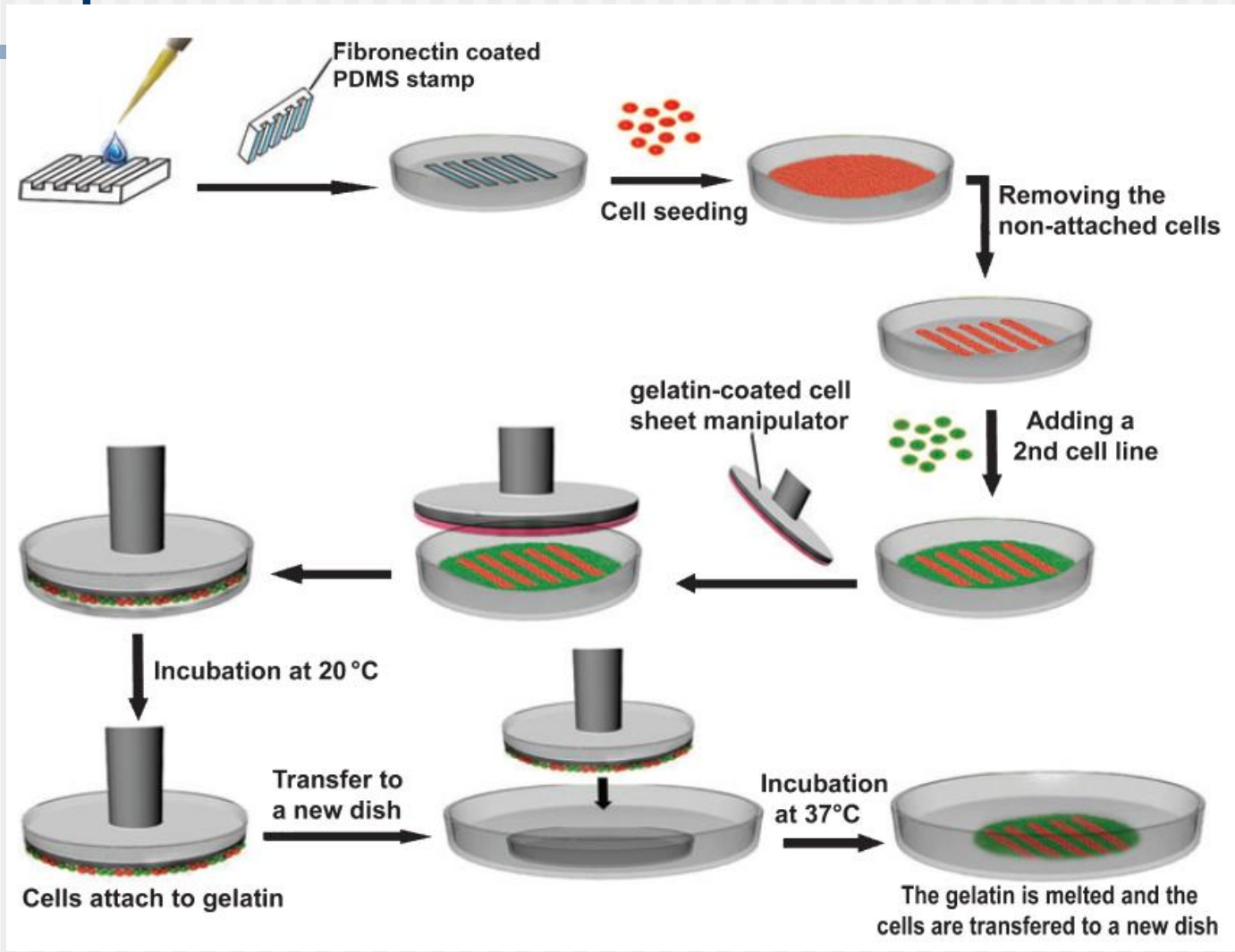
Micro-patterned-co-culture cell sheet



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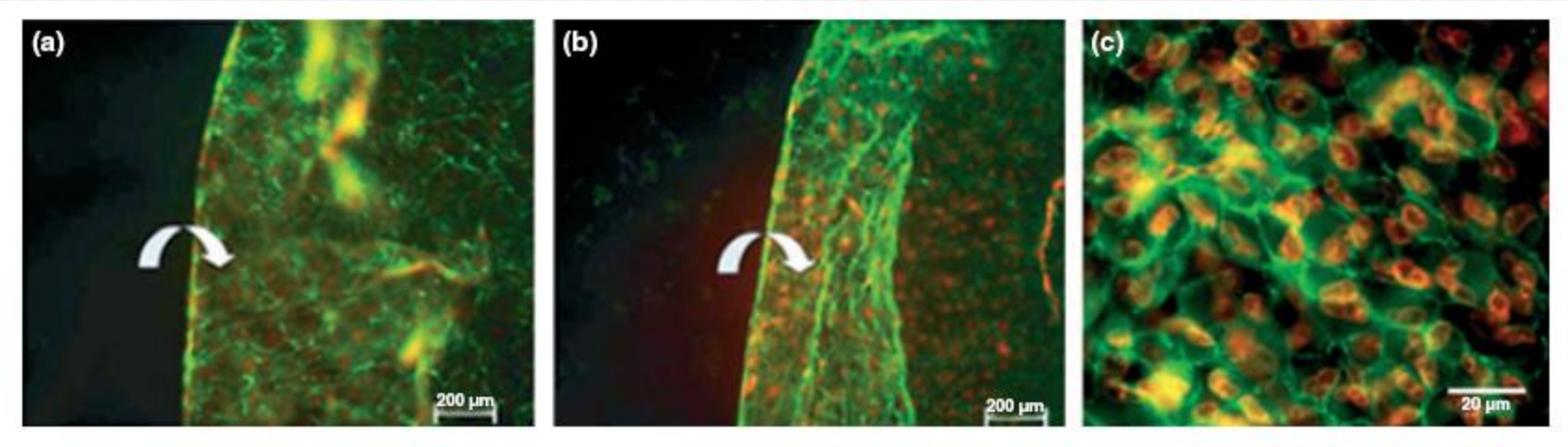
ECM is intact after detachment by CST



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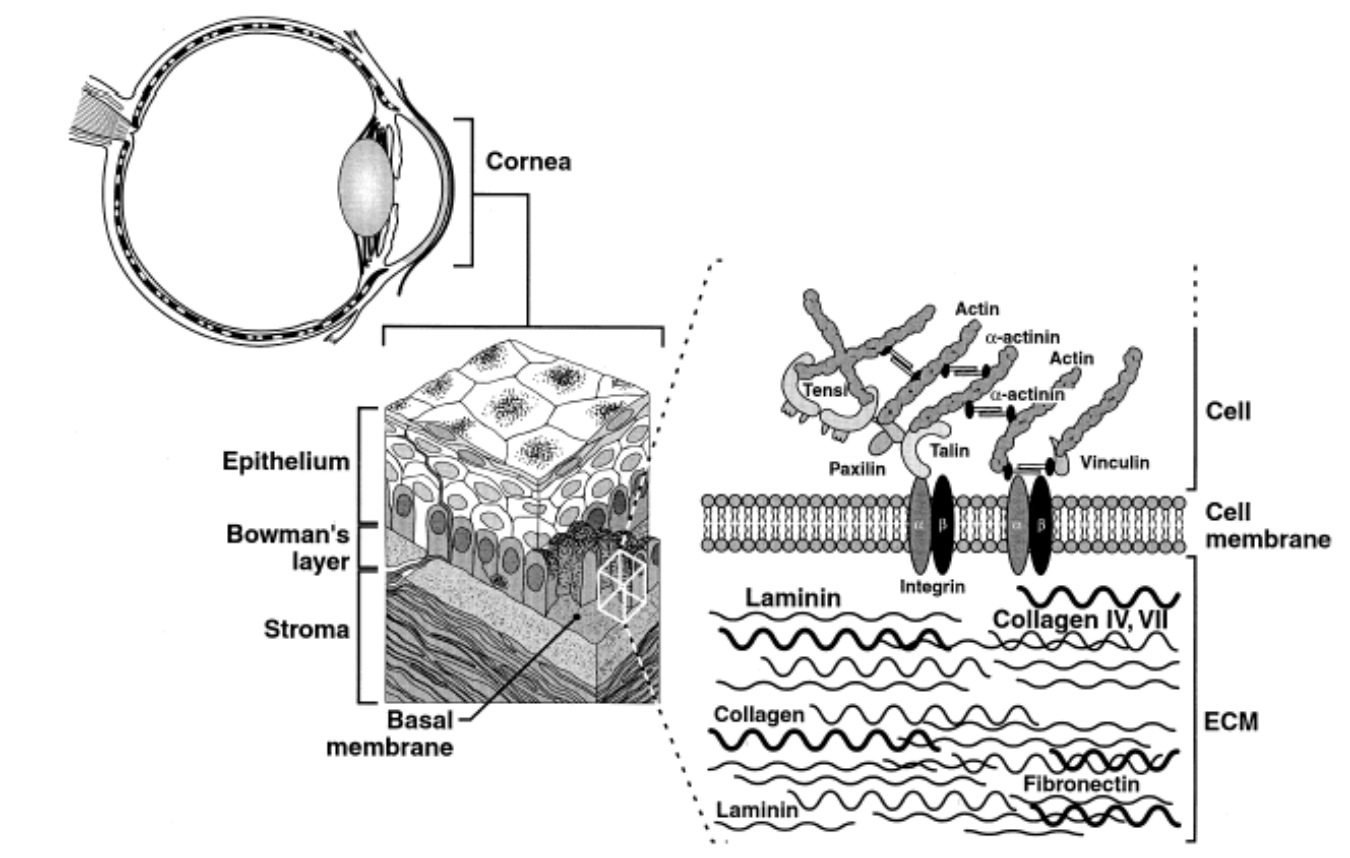
Corneal Equivalent

L. Germain et al. Progress in Retina and Eye Research 2000, 19(5):497-527



HUMAN CORNEA RECONSTRUCTION

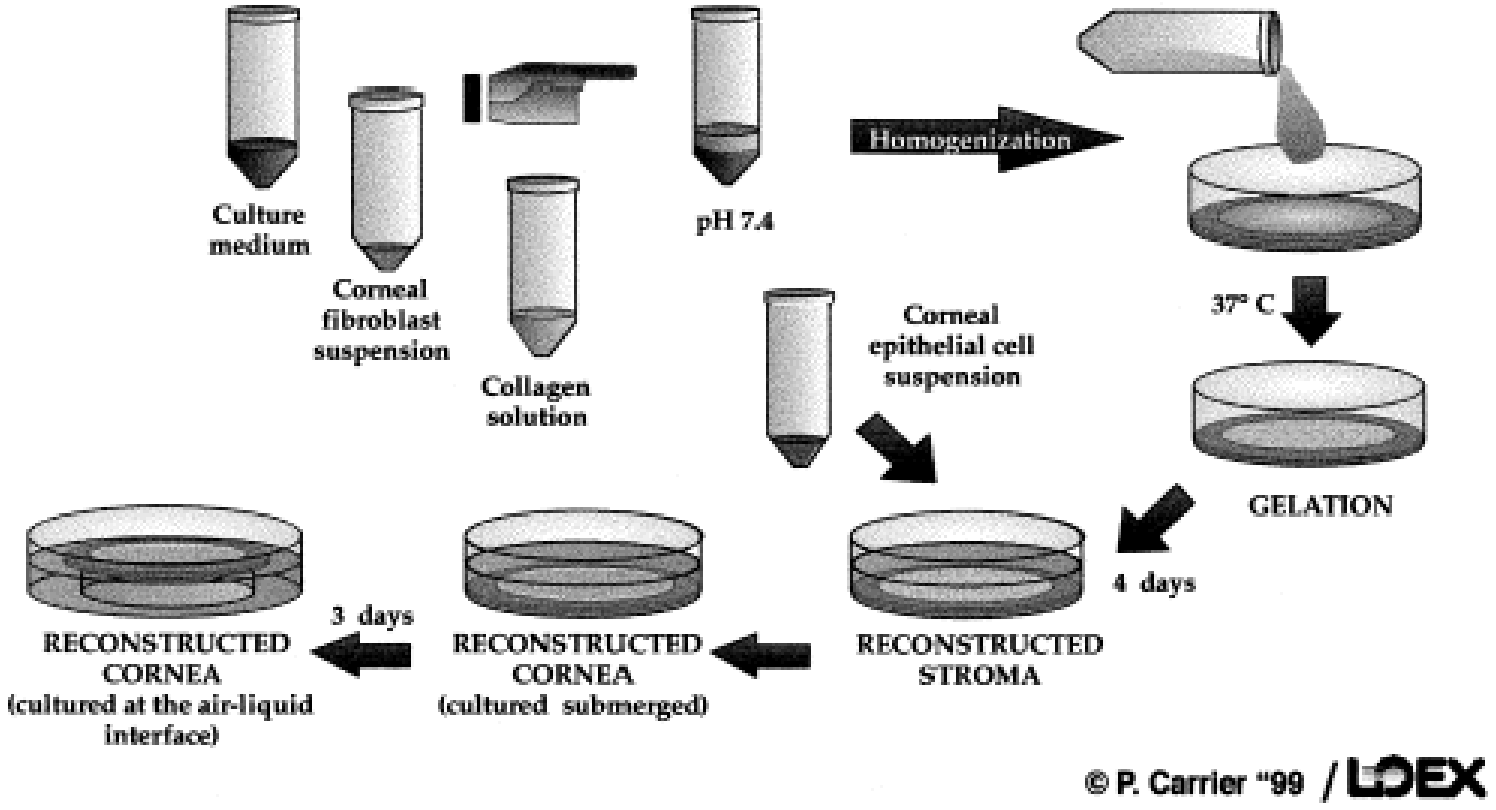


Fig. 4. Schematic representation of the production of reconstructed cornea.

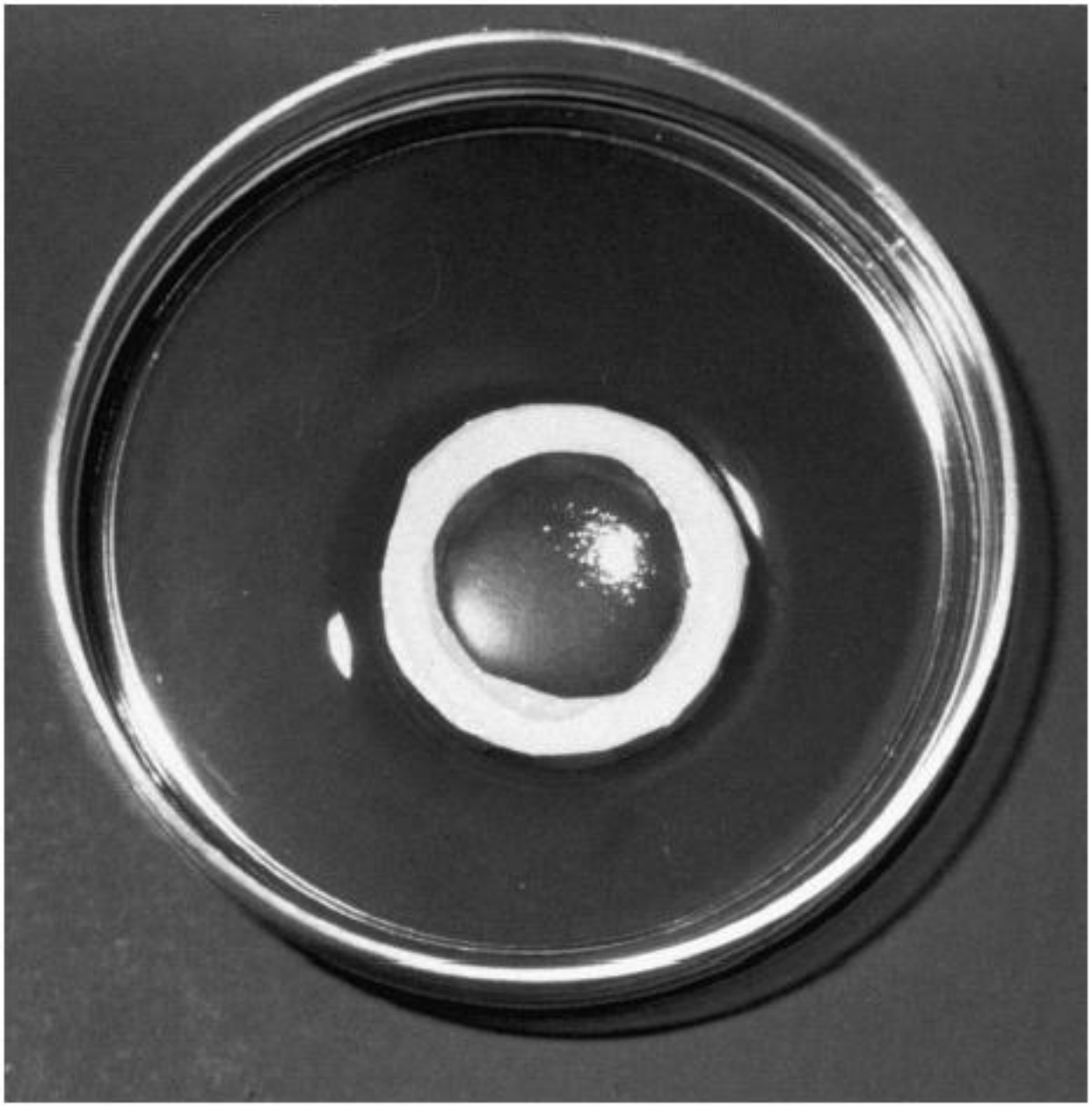


Fig. 7. Macroscopic view of the reconstructed cornea. The original colour figure has been published in *Science* (1999) **284**, 423.

Functional Human Corneal Equivalents Constructed from Cell Lines

May Griffith,[1]* Rosemarie Osborne,[2] Rejean Munger,[1] Xiaojuan Xiong,[1] Charles J. Doillon,[3] Noelani L. C. Laycock,[1] Malik Hakim,[1] Ying Song,[1] Mitchell A. Watsky[4]

Human corneal equivalents comprising the three main layers of the cornea (epithelium, stroma, and endothelium) were constructed. Each cellular layer was fabricated from immortalized human corneal cells that were screened for use on the basis of morphological, biochemical, and electrophysiological similarity to their natural counterparts. The resulting corneal equivalents mimicked human corneas in key physical and physiological functions, including morphology, biochemical marker expression, transparency, ion and fluid transport, and gene expression. Morphological and functional equivalents to human corneas that can be produced in vitro have immediate applications in toxicity and drug efficacy testing, and form the basis for future development of implantable tissues.

SCIENCE VOL 286 10 DECEMBER 1999

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- Collamer is 100% pure polymer – no residual monomers are present nor any viruses, bacteria or prions

What's in Collamer?

- 0.3% collagen - optimal biocompatibility
  - Less potential for damage to the iris, capsule, endothelium and other delicate anatomical structures
- Collagen has an affinity for fibronectin and attracts a monolayer on the surface of the lens
  - Fibronectin inhibits the deposition of other proteins
  - Once the monolayer is formed, a Collamer lens is no longer recognized as a foreign body

What's in Collamer? (continued-)

- Collamer seems to repair itself when exposed to YAG laser
- Collamer IOL is the same material as the ICL
- Lenses are tumble polished to assure a smooth edge finish

Table 1. Companies with HA or HA-derived products on the market or in development.

Company	Product
Amgen	Blend of HA with Interleukin-1 receptor antagonist
Anika	HA-*N*-acylurea derivatives for surgical applications (Incert®, Amvisc®), treatment of osteoarthritis (Orthovisc®, Hyvisc®), bone fracture healing (Ossigel™)
	HA products for ophthalmic and veterinary use
BioCoat	HA surface coating: Hydak™
Biomatrix	HA derivatives for viscosupplementation (Synvisc®), viscoprotection (Hylashield®), viscoaugmentation (Hylaform®) and others
Clear Solutions Biotechnology	HA hydrazide derivatives for cosmetic (Halosol™, HA-Quat™, Halogel™, Qualginate™, Halgin™, Halobeads™), medical (HA-Matricare™, Halosorb™), drug delivery (Hazomes-B2™, Cancept-HA™) or tissue engineering purposes (HA-Bed™)
Collaborative Laboratories	HA products in the cosmetic area: liposomes (Micasomes™ HOH) and specialty products (Botanigel™)
Fidia	HA ester derivatives for drug delivery and/or microsphere formulation
Genzyme	HA preparations for cosmetics (Hylucare®) or drug delivery (HyluMed®); Seprafilm®
Hyal Pharmaceutical Corp.	Combining HA with existing drugs: such as diclofenac (Hyanalgese™)
Kaken	HA products for osteoarthritis treatment
Pharmacia & Upjohn	HA products for ophthalmology: Healon
Seikagaku Corp.	HA-enzyme conjugates
Shiseido Company, Ltd	HA products for cosmetics and drug delivery
SurModics Inc. (formerly BSI Corp.)	HA surface coating using PhotoLink® technology
Telios Pharmaceuticals, Inc. (Integra Life Sciences Corp.)	HA hydrogels for tissue engineering

Restylane

RESTYLANE vs. other treatments

	Description	Procedure	Safety	Duration
Stabilized Non-Animal Hyaluronic acid gel (NASHA)	Hyaluronic acid is a substance that exists naturally in the body. Its most important function is to carry and bind water. Non-animal, stabilized hyaluronic acid (NASHA) does not contain animal protein and does not require a skin test prior to treatment. NASHA is a unique form of hyaluronic acid, manufactured under the name of RESTYLANE.	RESTYLANE is designed for different purposes and effects, from reducing wrinkles and folds to enhancing facial contours and sculpting lips. The treatment often takes less than half an hour.	Injection-related adverse reactions such as swelling, redness and tenderness might occur. Other adverse reactions are very rare.	The result is immediate and can last from 6 - 12 months, depending on the patient and the area that is being treated. Wrinkle treatment tends to last from 6 - 12 months and lip enhancement for around 6 months.
Other Hyaluronic acid products	In addition to RESTYLANE, a range of hyaluronic acid treatments is available. Some of these products are derived from cockscombs bred especially for this purpose.	Hyaluronic acid is used to treat lips, wrinkles and facial lines. The treatment takes less than an hour to perform.	Swelling, redness and tenderness may occur at the site of injection. Other adverse reactions have been reported to occur.	The duration varies depending on the type of product, the condition of the patient and the area being treated.

Description	Procedure	Safety		Duration
Botulinum Toxin Type A	Botulinum Toxin Type A is a toxin that blocks neuromuscular transmission when injected in tiny amounts into specific muscles to treat and improve lines and wrinkles associated with facial expression. Manufactured under the names of Botox© and Dysport©	Botulinum toxin is injected in minute quantities. It blocks the nerve impulses to the facial muscles responsible for contraction, thereby relaxing the muscles and smoothing facial lines. It is most effective on frown lines and crow's feet around the eyes. Accurate dosing and precise positioning are critical in order to freeze the right muscles just enough. The treatment takes about 30 minutes and the effect of a treatment can be seen within 1 - 2 weeks.	The long-term risks of botulinum toxin are unknown. Redness may occur, as well as bruising, numbness, swelling and headaches. If overdosed or the injection is performed incorrectly, the patient can be left with an immobile face or droopy eyelids until the effect of the injection wears off.	The result lasts for about four months.
Collagen treatment	Collagen is derived from the hides of cows specially bred for the purpose. Collagen can also be derived from pig skin. Because collagen is an animal product, a collagen test skin implant is administered to determine whether the patient is allergic to the implant, four weeks prior to the treatment. Collagen is manufactured under many names, two of which are Zyderm and Zyplast.	Collagen can be injected under the skin to fill lines and wrinkles. It is also used for lip enhancement. The treatment takes less than an hour.	Redness and swelling may occur. Allergic reactions are possible in 3-4% of patients.	The effect normally lasts for three to four months.
	of Artecoll, Dermalive, NewFill, Evolution, Outline and Amazingel.		skin discoloration or granulomas.	
Fat injections / liposculpture	Fat injections or liposculpture are performed using the patient's own fat cells (autologous fat) taken from unwanted fat deposits. There are many variables in this treatment, including the way the fat is extracted, whether frozen or fresh fat is used, the area that is injected, the amount of fat that is used and how deeply the fat is injected.	The fat is injected under the skin to treat hollow cheeks, to enhance lips or cheeks or to treat deep folds. The procedure is performed under local anaesthesia and can take from less than one hour to several hours.	Some lumpiness can occur, in addition to injection-related reactions. If the patient puts on weight, the injected area can become enlarged, which may not be desirable.	The reported duration varies from a few months to several years.
Permanent/semi-permanent skin implants	Permanent and semi-permanent skin implants contain a range of different synthetic ingredients to make the product last longer. Permanent and semi-permanent skin implants are manufactured under the names of Artecoll, Dermalive, NewFill	These products are used to fill lines and wrinkles and to augment lips by injection. Some of the products require at least two treatment sessions.	For some of the products, very little or no safety data at all are available. Immediate reactions include redness and swelling at the injection site. Longer-term reactions are also known to occur over time. They include permanent redness, inflammation, lumpiness,	The duration differs between the products. Some claim to last for ever, while others offer a sustained improvement for up to twelve months.

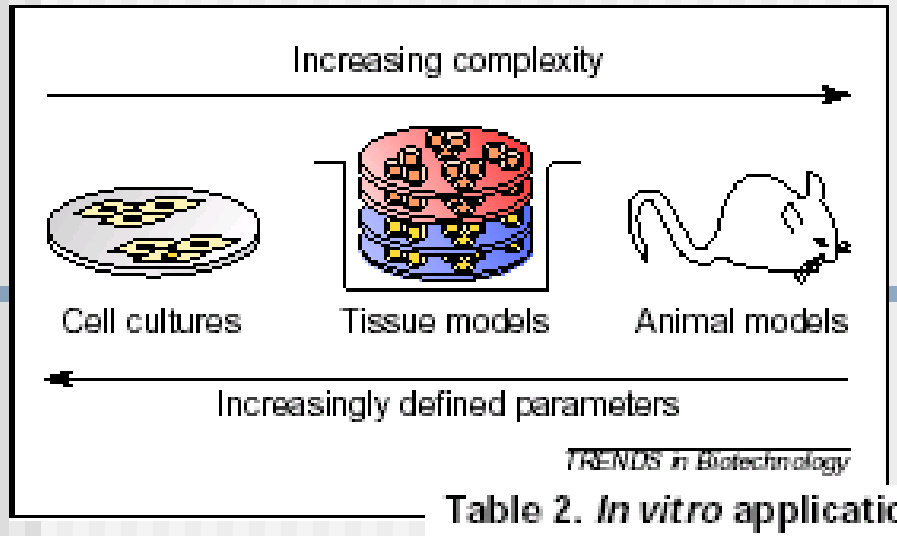

Table 2. *In vitro* applications of 3D tissue models

Application	Example
In vitro assay	Test system for drugs, cytokines, morphogenetic factors and enzyme inhibitors
Morphogenesis model	Induction of proliferation and differentiation in an interactive 3D culture
Establishment of tissue transplants	Combination with scaffolds as supportive structures
Angiogenesis model	Endothelial cells interacting with tumor cells, inflammatory cells and so on
Cell migration	Migration of mononuclear cells, fibroblasts and so on in an extracellular matrix, chemotaxis, cell adhesion, homing and infiltration
Immunological studies	Interaction of T cells with macrophages, antigen presenting cells and fibroblasts in context of the extracellular matrix
Genetically altered cells	Transfection of mesechymal stem cells for the expression of morphogens and interaction with resident cells