Biodegradable Copolymers for Tissue Engineering: Preparation and Characterization and Cell Growth

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TÜBİTAK

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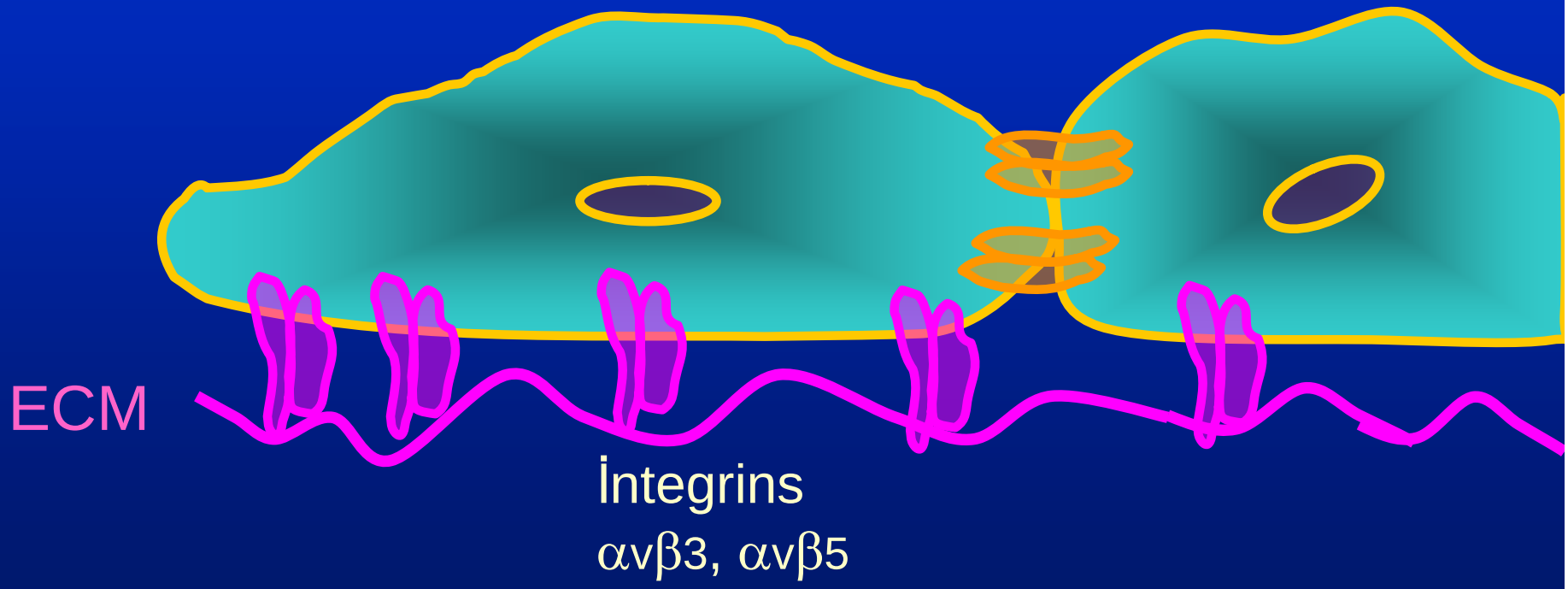
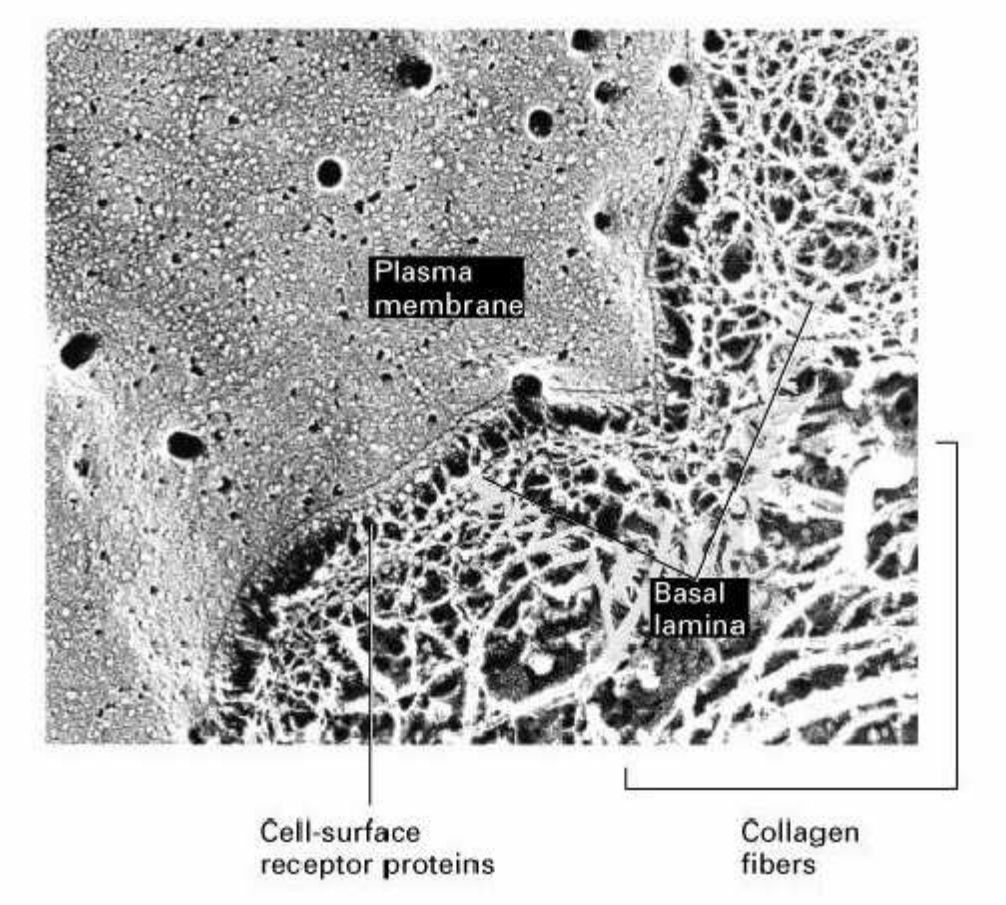
Cell-cell and Cell extracellular matrix interactions

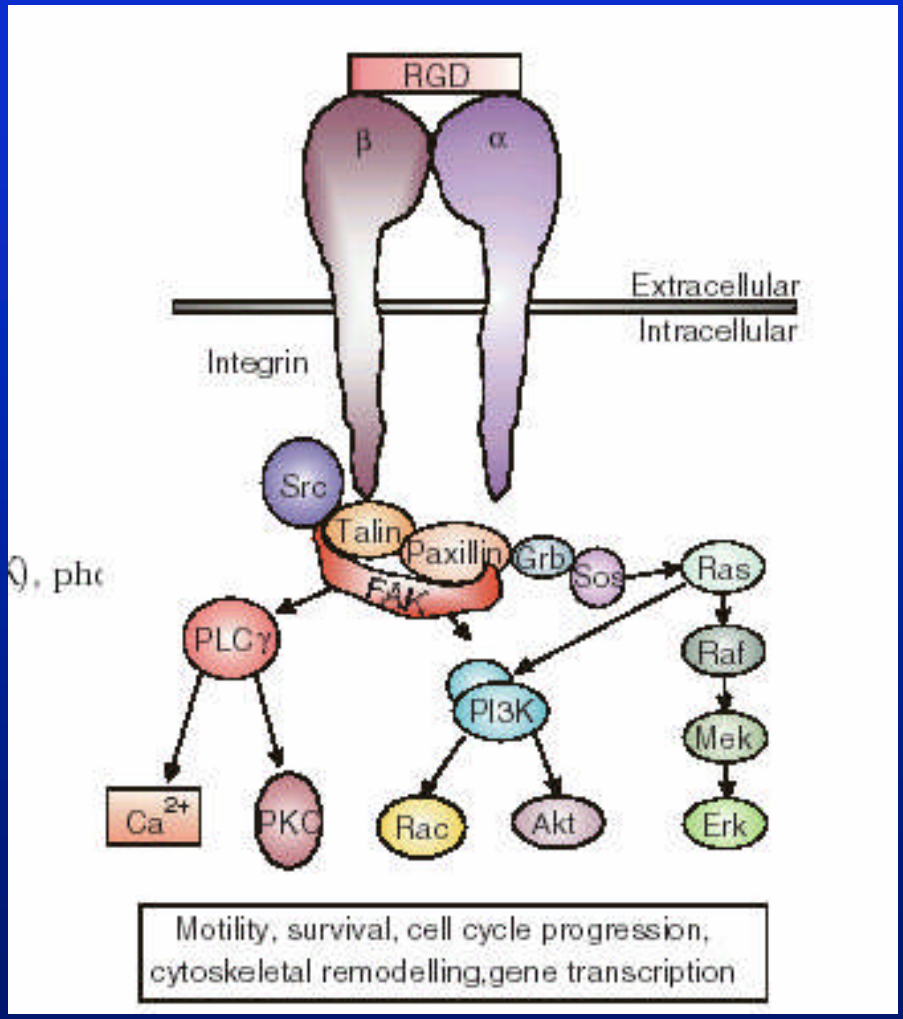


A cell interacting with the basal membrane formed by type IV collagen and laminin



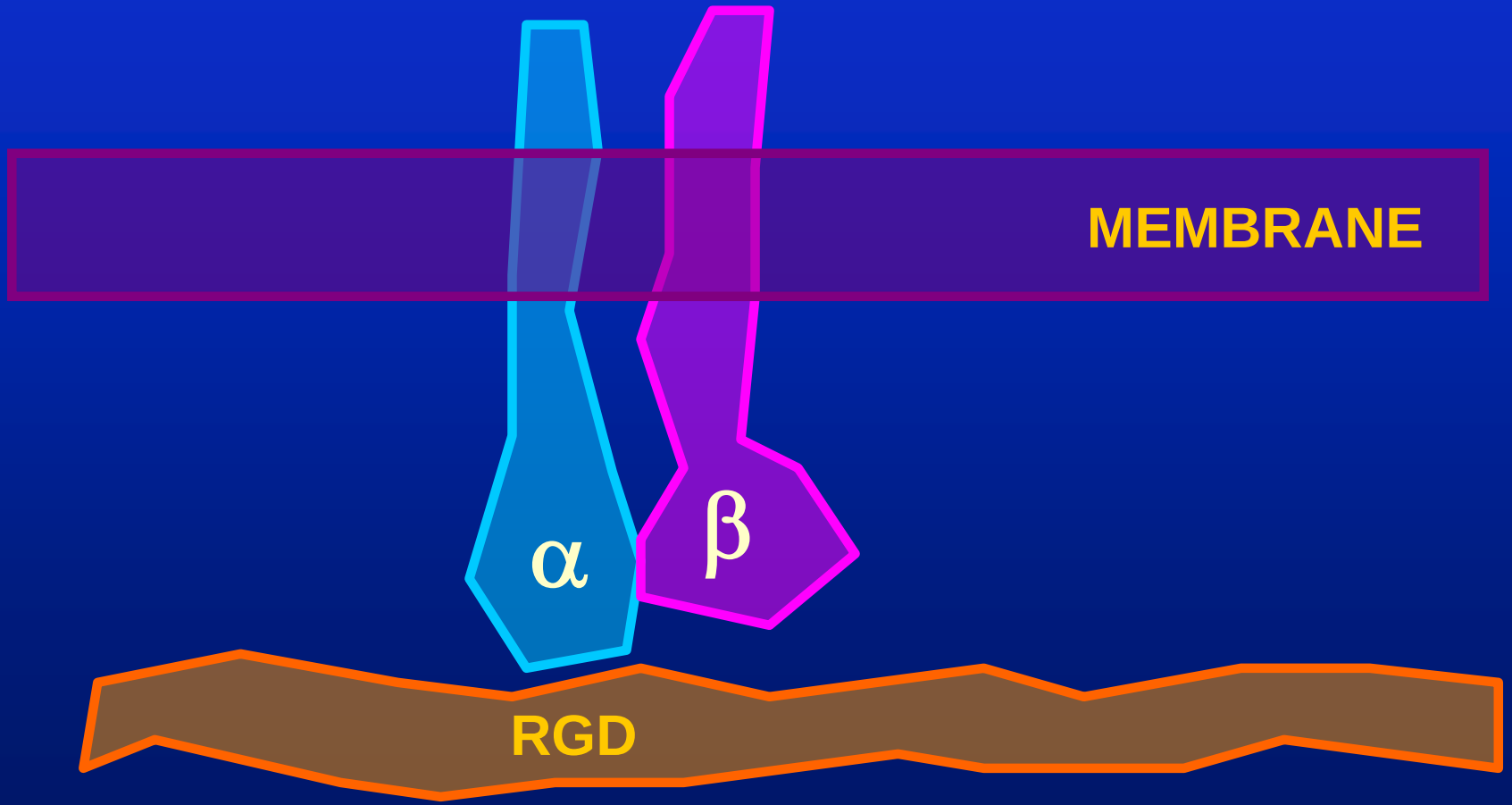
Prof. Anne Ridley

Signal Transduction from Integrin heterodimers



J. T. Price & E. W. Thompson *Expert Opin. Ther. Targets* (2002) 6(2):217-233

Integrin heterodimers



Synthetic Polymers As Tissue Engineering Scaffolds

1)Poly(L-lactic acid-co-ethylene oxide-co-aspartic acid) terpolymer (PLLA/PEO/Pasp)

2)Poly(ethylene glycol)-poly(D,L-lactide-co-glycolide)-(polyethylene glycol) triblock copolymers modified with collagen

Synthesis of PLLA/PEO/PAsp Terpolymer

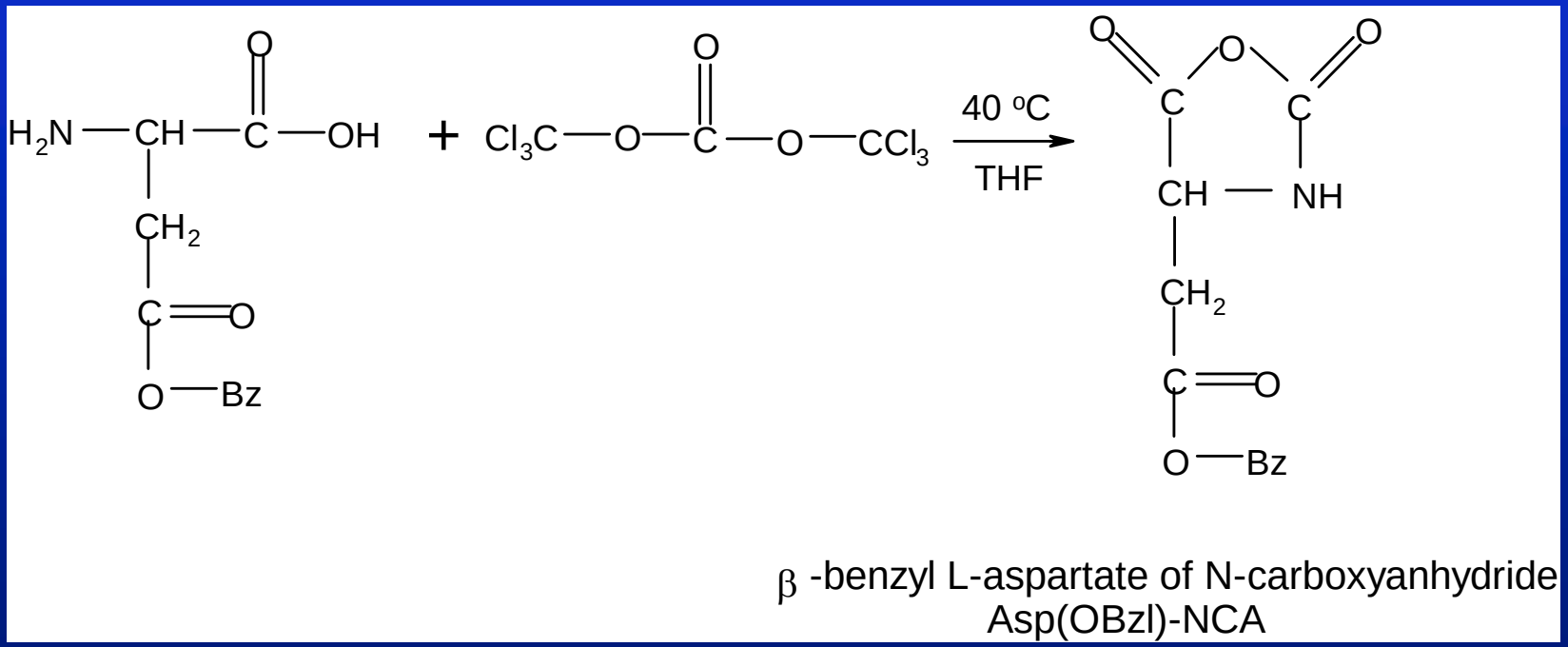
First step is synthesis of N-carboxy anhydride of β-benzyl L-aspartate

β-benzyl L-aspartate + triphosgene

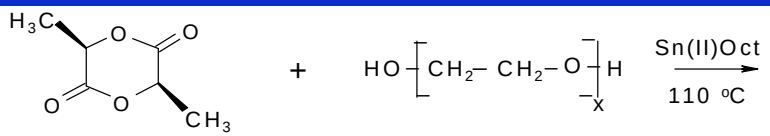
↓

40°C, THF

β-benzyl L-aspartate of N-carboxy anhydride Asp(OBzl)-NCA

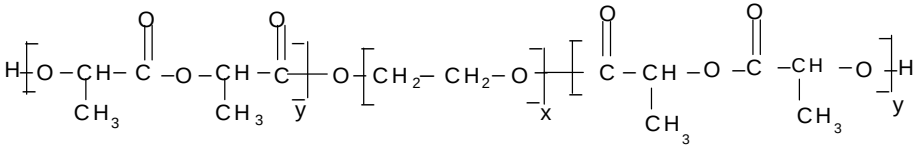


β -benzyl L-aspartate of N-carboxyanhydride
Asp(OBzl)-NCA

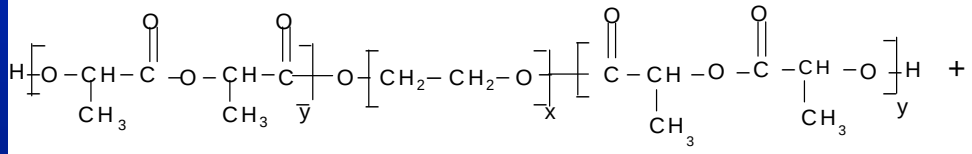


L-Lactide

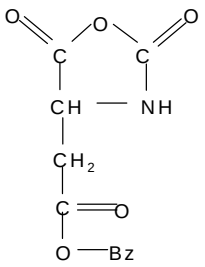
poly(ethylene oxide)



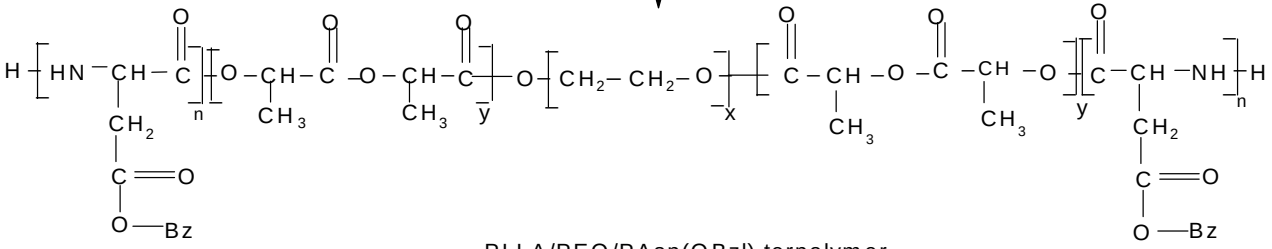
PLLA-PEO-PLLA macromer



PLLA-PEO-PLLA macromer



Asp(OBzl)-NCA



PLLA/PEO/PAsp(OBzl) terpolymer

Characterization

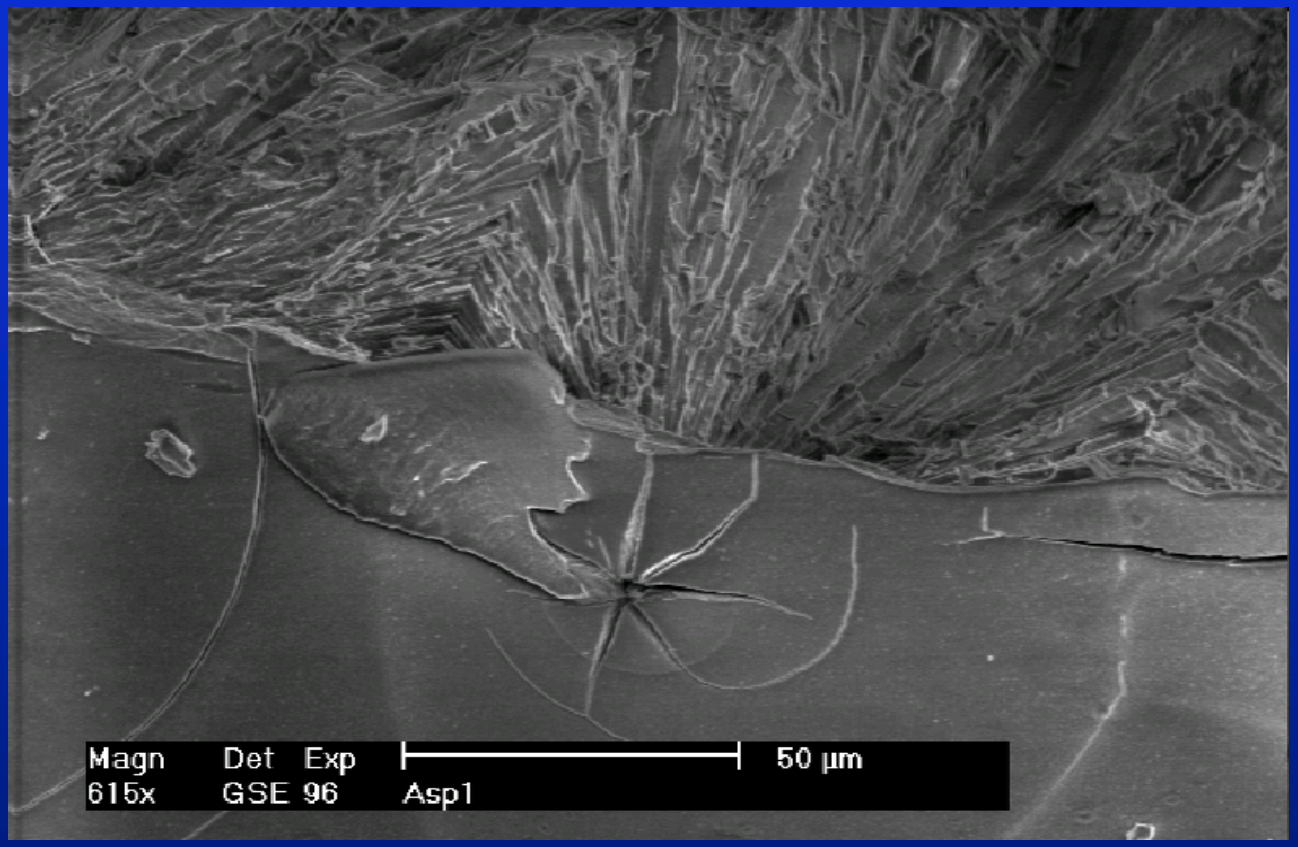
1) FTIR(Fourier Transform Infrared Spectroscopy)

2) ^{1}H-NMR (Nuclear Magnetic Resonance)

3) ESEM (Environmental Scanning Electron microscope)

4) SEC (Size Exclusion Chromatography)

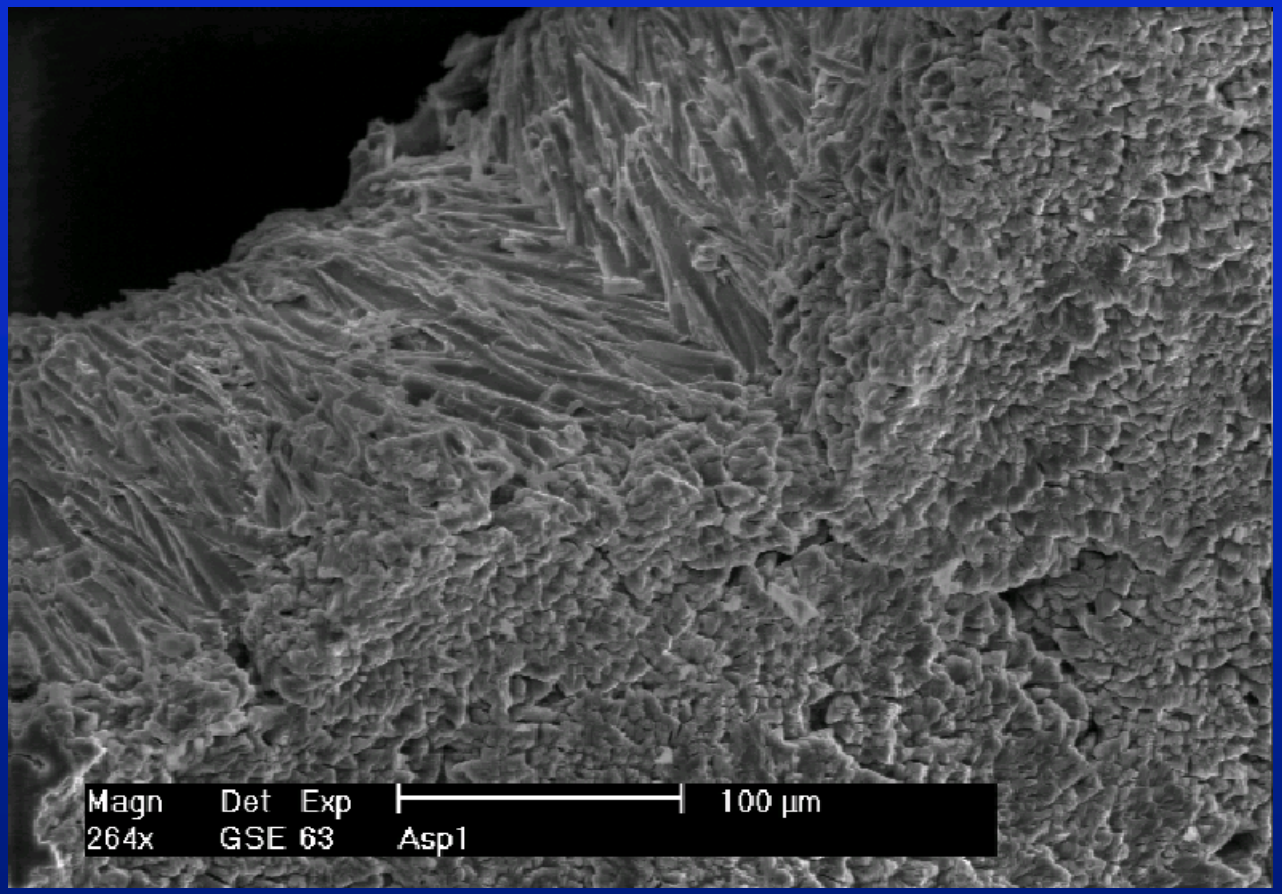
Polymer	M_n (g/mol)	M_w (g/mol)	P.D.
PLLA/PEO/PLLA	4600	7300	1.58
PLLA/PEO/PAsp(OBzl)	6500	8750	1.35
PLLA/PEO/PAsp	5200	6950	1.34



ESEM micrographs of cross-section and side surface details of PLLA/PEO/PAsp terpolymer

A star shaped fracture zone on the polymer microsection is seen, adjacent to the locus of failure.

ESEM photos: Zulal Mısırlı, Boğaziçi Univ. Advanced Technologies R&D Center.



ESEM micrographs of fractured surface details of PLLA/PEO/PAsp terpolymer

Fractured surface has an oriented texture. This is probably due to the semicrystalline nature of the polymeric sample.

The results of characterization showed that;

1) PLLA/PEO/PAsp is crystalline

2) Its surface structure is brittle

Due to its inherent brittle behaviour, the terpolymer has been blended with a high molecular weight copolymer (PLGA) in two different compositions:

(1) (PLLA/PEO/PAsp)/PLGA 25/75 (w/w) blend

(2) (PLLA/PEO/PAsp)/PLGA 50/50 (w/w) blend

Synthesis of Poly(L-lactic-co-glycolic acid) Copolymer

- Ring Opening Polymerization
- Catalyst:Sn(II)Oct.
- Monomer/Catalyst, mole ratio, 1/1000
- The feed molar ratio of the L-lactide/glycolide= 85/15
- Rxn temperature: 115°C
- Rxn time: 24 hours
- Mw: 95000 g/mol

Porous Film Fabrication

-Solvent casting particulate leaching technique

-Polymers (PLGA 85/15, (PLLA/PEO/PAsp)/PLGA in two different compositions (25/75; 50/50))

-Polymers/dissolved in chloroform

-Polymer/salt ratio:0.18

-1ml of polymer solution was placed into Teflon molds (d:22mm, h:10mm) packed with NaCl particles(size:200-400µm).

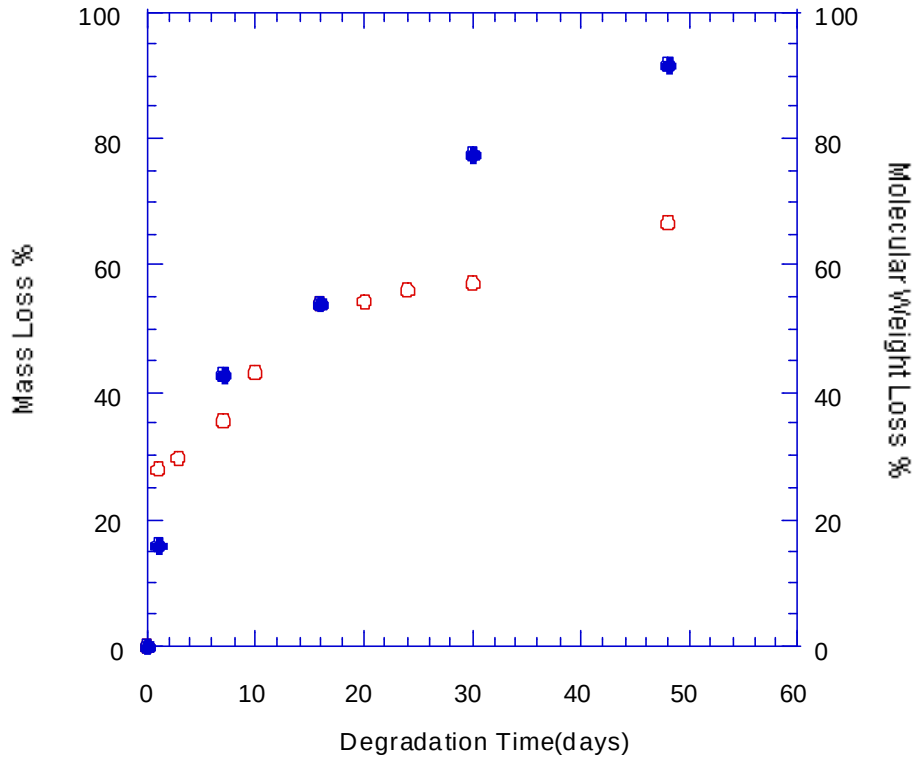
-Solvent allowed to evaporate

-Entrapped salt particles were removed by immersing the films in distilled water for 48 hour.

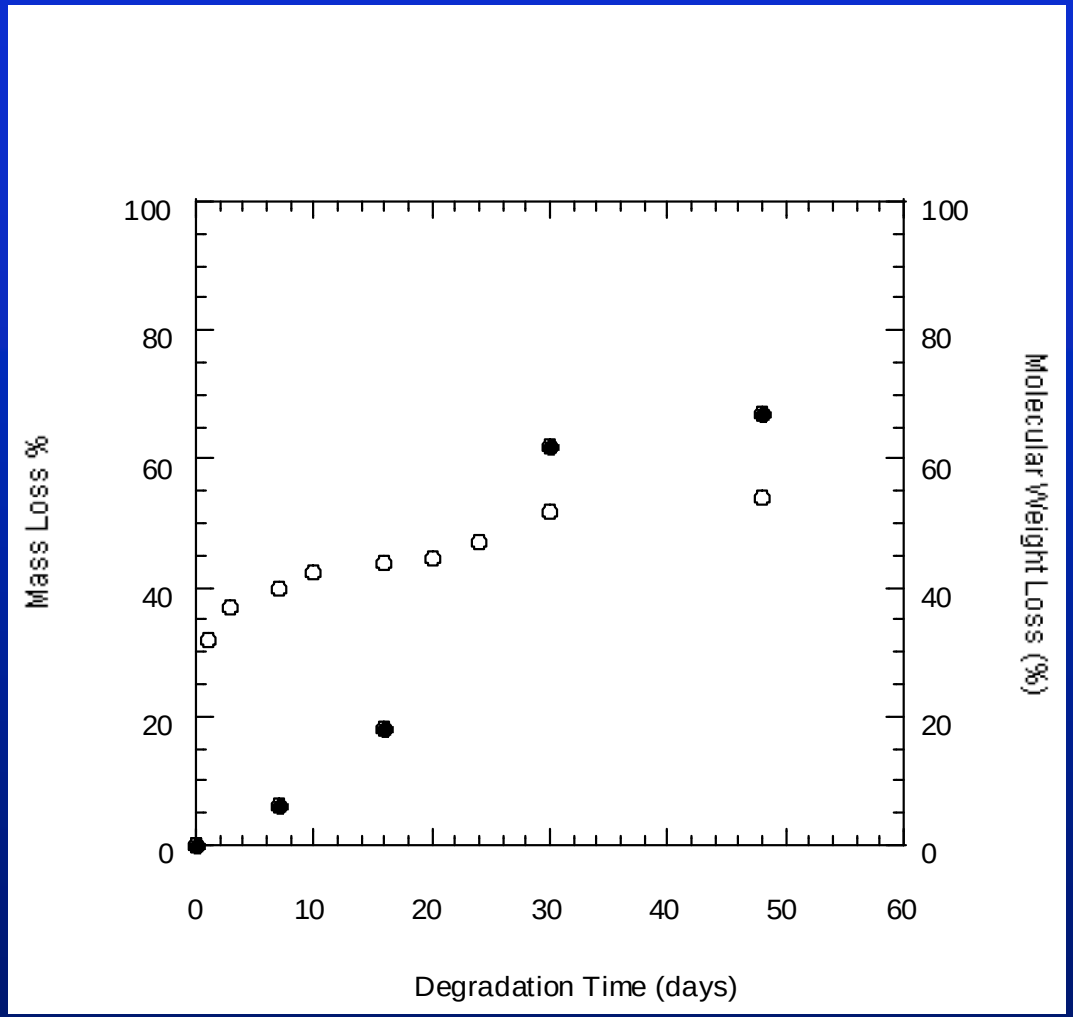
Degradation

The hydrolytic degradation of the porous polymeric mixtures ((PLLA/PEO/PAsp)/PLGA; 25/75 and 50/50) were investigated in PBS at pH=7.4 37°C

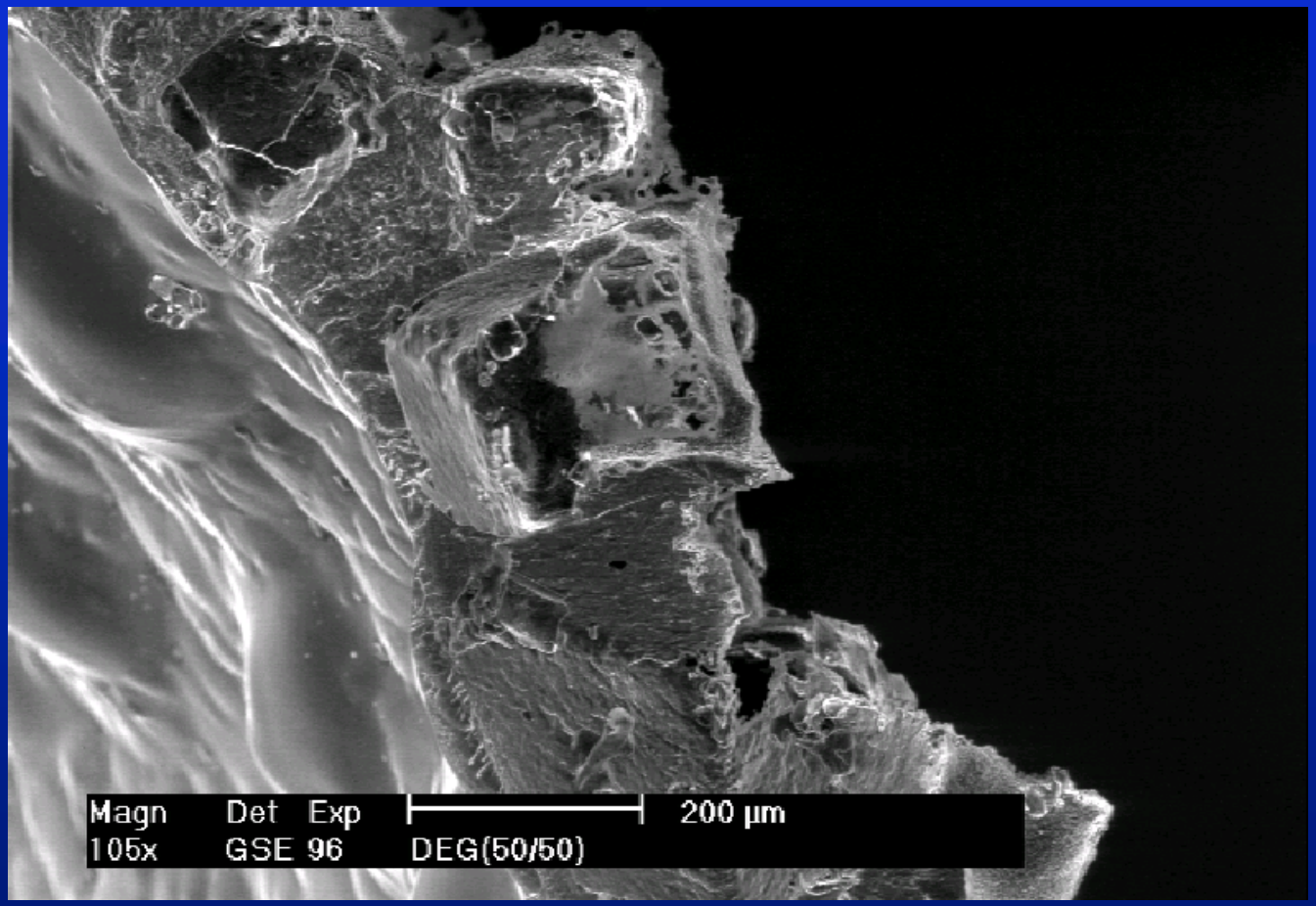
Figure 5



Mass and Molecular weight loss for the 25/75 (PLLA/PEO/PAsp)/PLGA mixture: (o) mass loss; (•) Mw loss

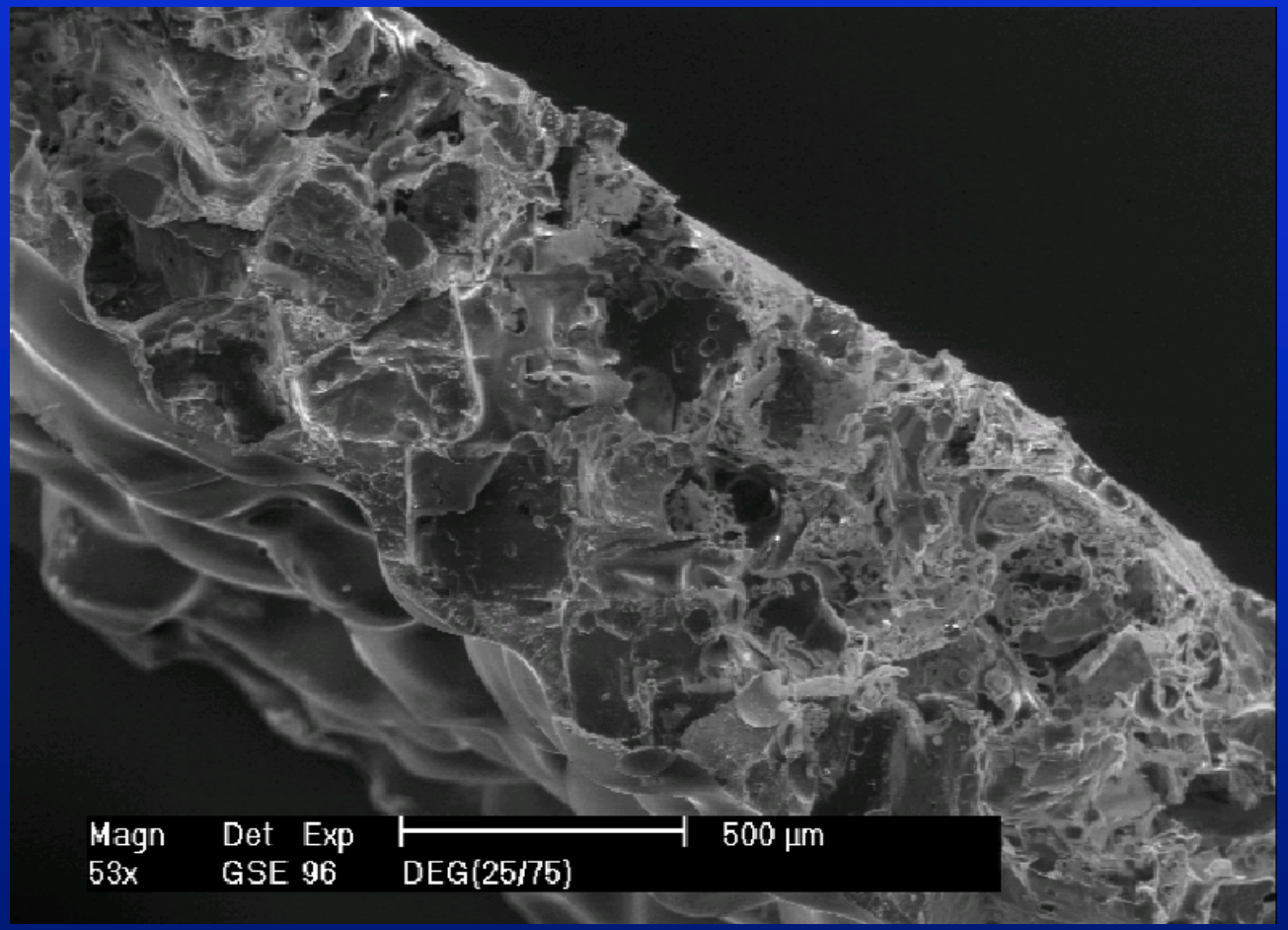


Mass and Molecular weight loss for the 50/50 (PLLA/PEO/PAsp)/PLGA mixture: (o) mass loss; (•) Mw loss

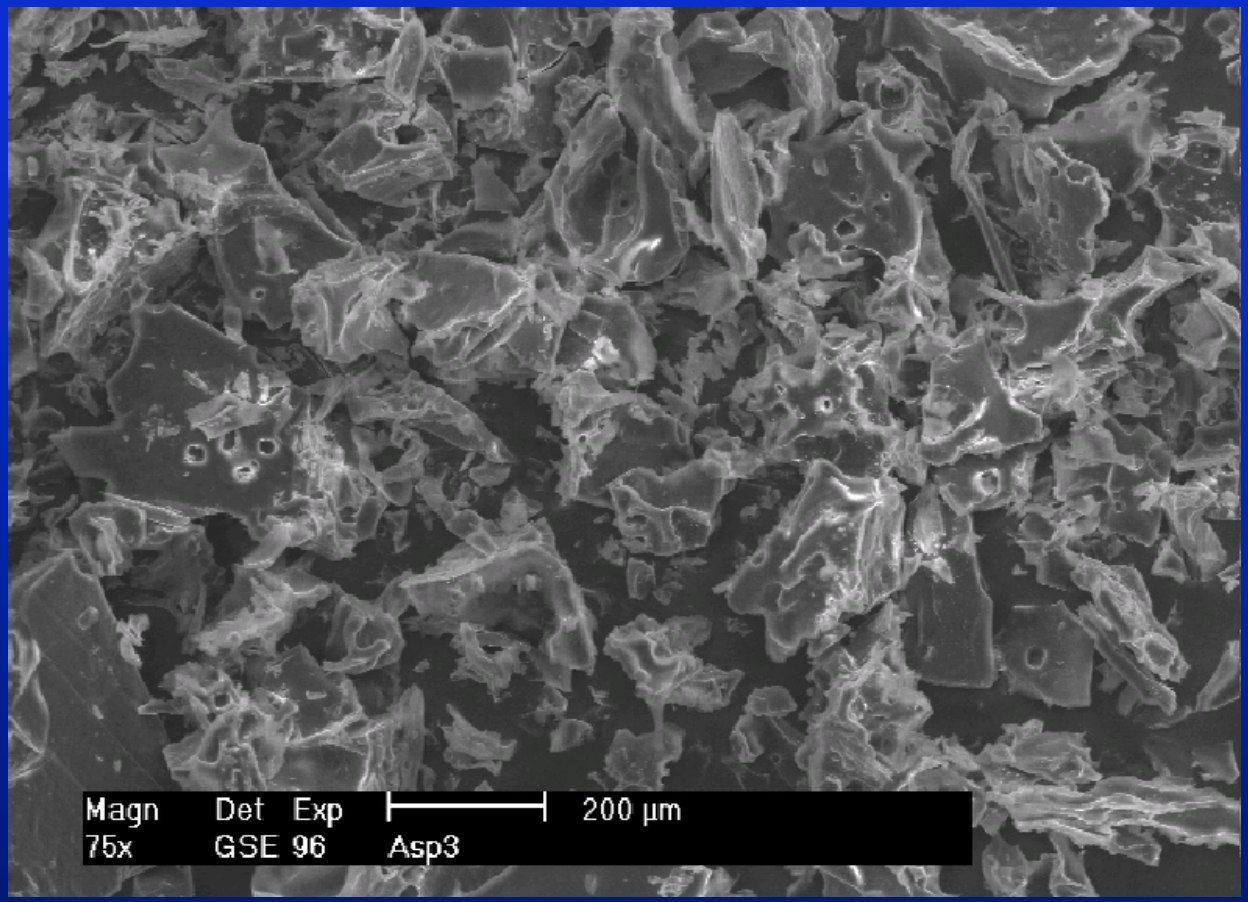


ESEM micrograph of (PLLA/PEO/PAsp)/PLGA 50/50 blend before degradation

ESEM photos: Zulal Mısırlı, Boğaziçi Univ. Advanced Technologies R&D Center.



ESEM micrograph of (PLLA/PEO/PAsp)/PLGA 25/75 blend before degradation



ESEM micrograph of (PLLA/PEO/PAsp)/PLGA 50/50 blend after degradation of 10 days.

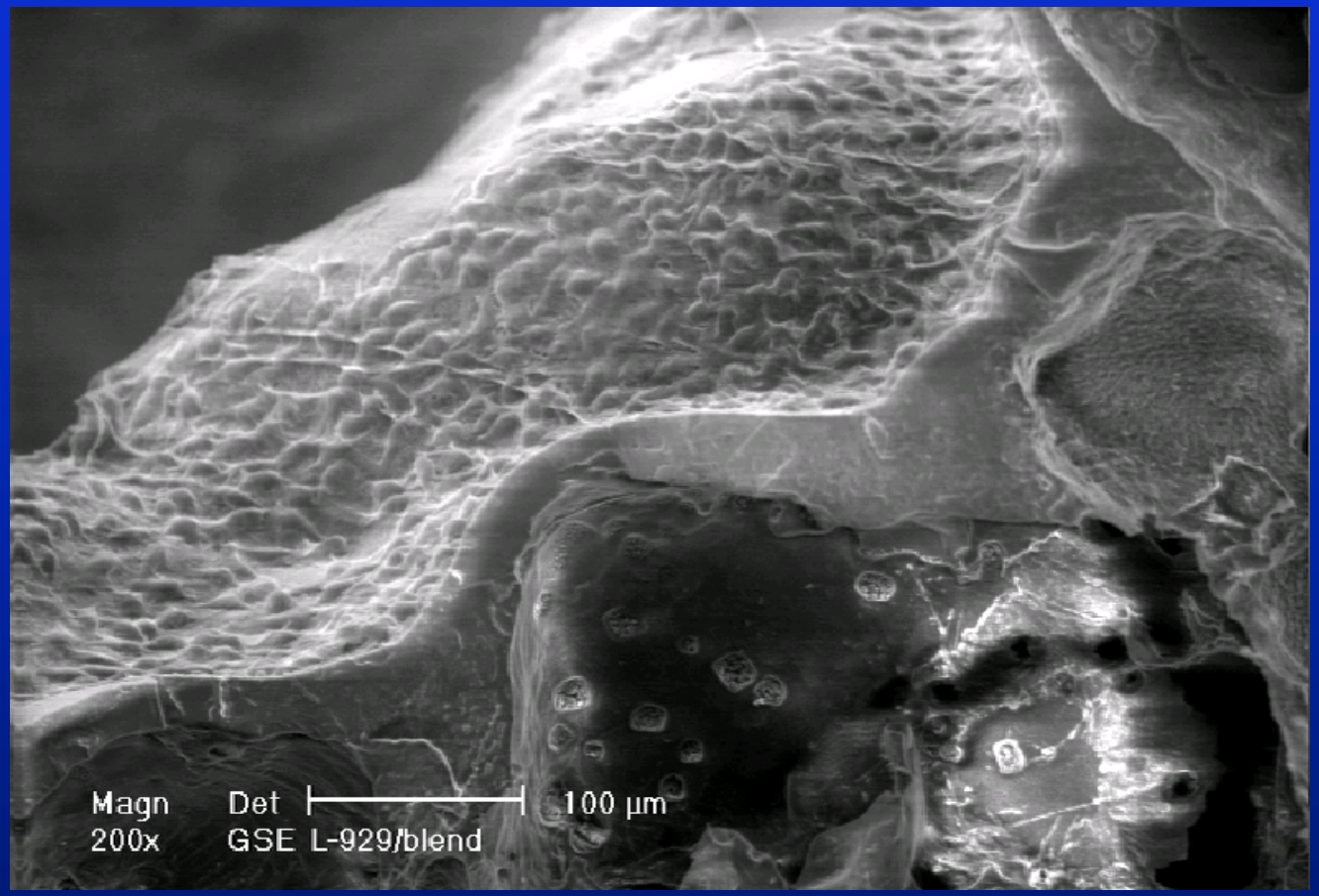
Neutral Red cell viability assay

- An experiment in which cell viability was measured and compared on different materials.

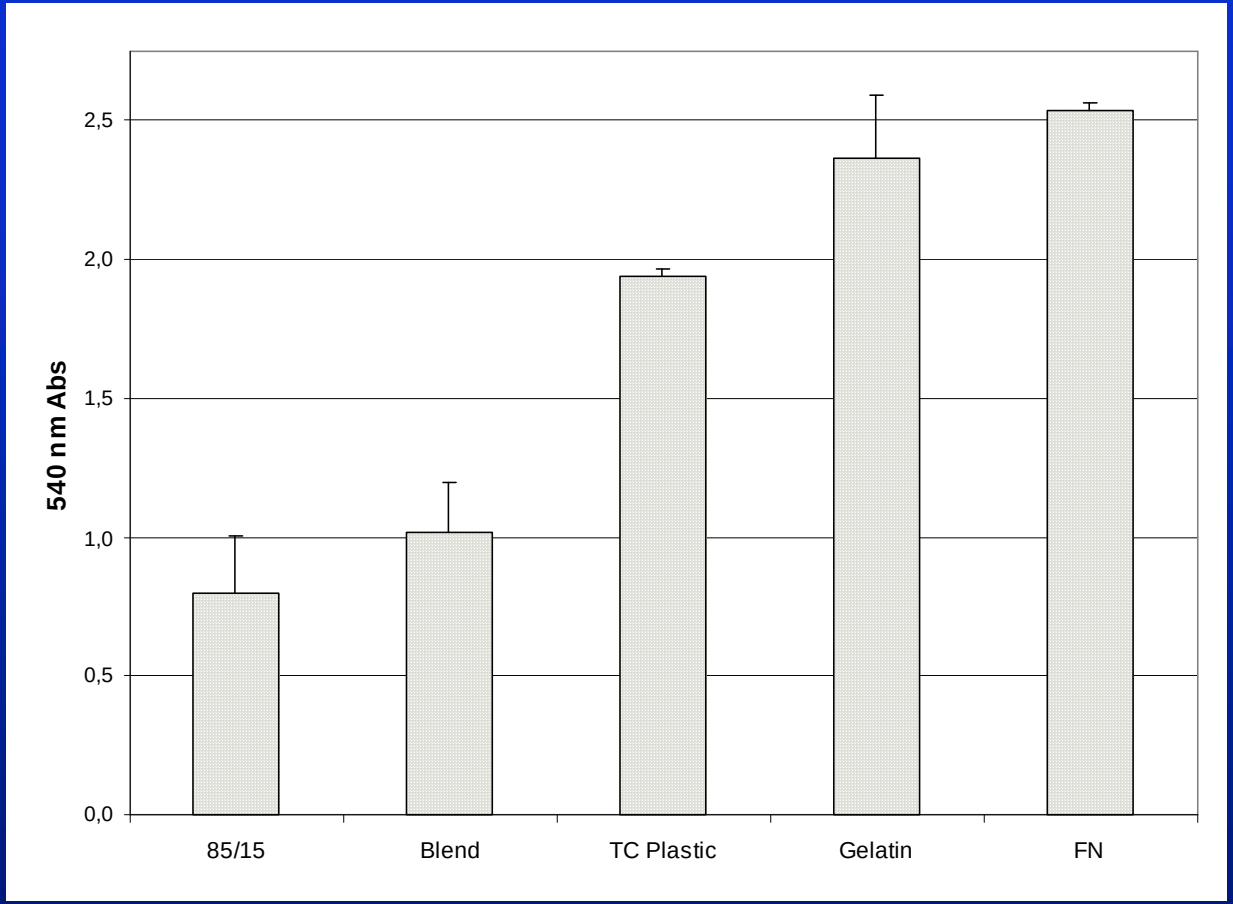
- L929 cells placed on TC Plastic, gelatin or fibronectin rapidly proliferate, giving high absorbance values after 72 hours.

- PLGA (85/15) copolymer or (PLLA/PEO/PAsp)/PLGA 25/75 blend allowed the attachment and proliferation of a significant amount of cells.

- The number of L929 cells on the blend was slightly higher than the 85/15 PLGA copolymer.



ESEM micrograph showing the cross section of the 25/75 blend 72 hours following seeding with L929 cells, showing a group of L929 cells attached to a surface inside a pore in the blend structure as well as the outer surface.



Cell viability assay by neutral red uptake. 150,000 L929 cells were seeded per 22 mm diameter polymer surface or glass coverslip and grown for 72 hours in a CO_2 incubator. TC Plastic; tissue culture plastic, FN;fibronectin, 85/15; Poly(L-lactic-co-glycolic acid) copolymer, Blend;(PLLA/PEO/PAsp)/PLGA 25/75 mixture

Conclusion

ESEM results showed that PLLA/PEO/PAsp is crystalline and its surface structure is brittle.

- The hydrolytical degradation of polymeric blends was performed up to 48 days and the results indicated that the degradation occurs by chain scission.

- For the 25/75 (PLLA/PEO/PAsp)/(PLGA) sample, the mass loss and molecular weight loss is faster than the 50/50 (PLLA/PEO/PAsp)/(PLGA) sample because of the high (PLGA) content in the mixture.

- The cell seeding experiments showed that cell growth supporting characteristics of the 25/75 (PLLA/PEO/PAsp)/(PLGA) blend was slightly higher than the 85/15 PLGA copolymer which was used in producing the blend.

2) Synthesis of poly(ethylene glycol)-poly(D,L-lactide-co-glycolide)-poly(ethylene glycol) triblock copolymer PEG-PDLLG-PEG, and its modification with type I collagen.

The synthesis consists of several steps:

1. Transformation of the PEG carboxylic groups into more reactive acyl halide groups,
2. Coupling reaction with OH terminated PDLLG,
3. Activation of PEG-PDLLG-PEG with N-hydroxysuccinimide to produce a polymer more reactive to proteins and peptides,
4. Attachment of type I collagen to the PEG-PDLLG-PEG copolymer.

Preparation of PEG-coupled PDLLG includes two steps:

1) Bis carboxy methyl PEG (PEG-COOH) was reacted with thionyl chloride producing highly reactive bis-chlorocarboxy methyl PEG (PEG-COCl)
2) Reacting bis-chlorocarboxymethyl PEG with OH terminated PDLLG produced carboxyl terminated PEG-PDLLG-PEG triblock copolymer.

Activation of PEG-PDLLG-PEG triblock copolymer with N-hydroxysuccinimide (NHS):

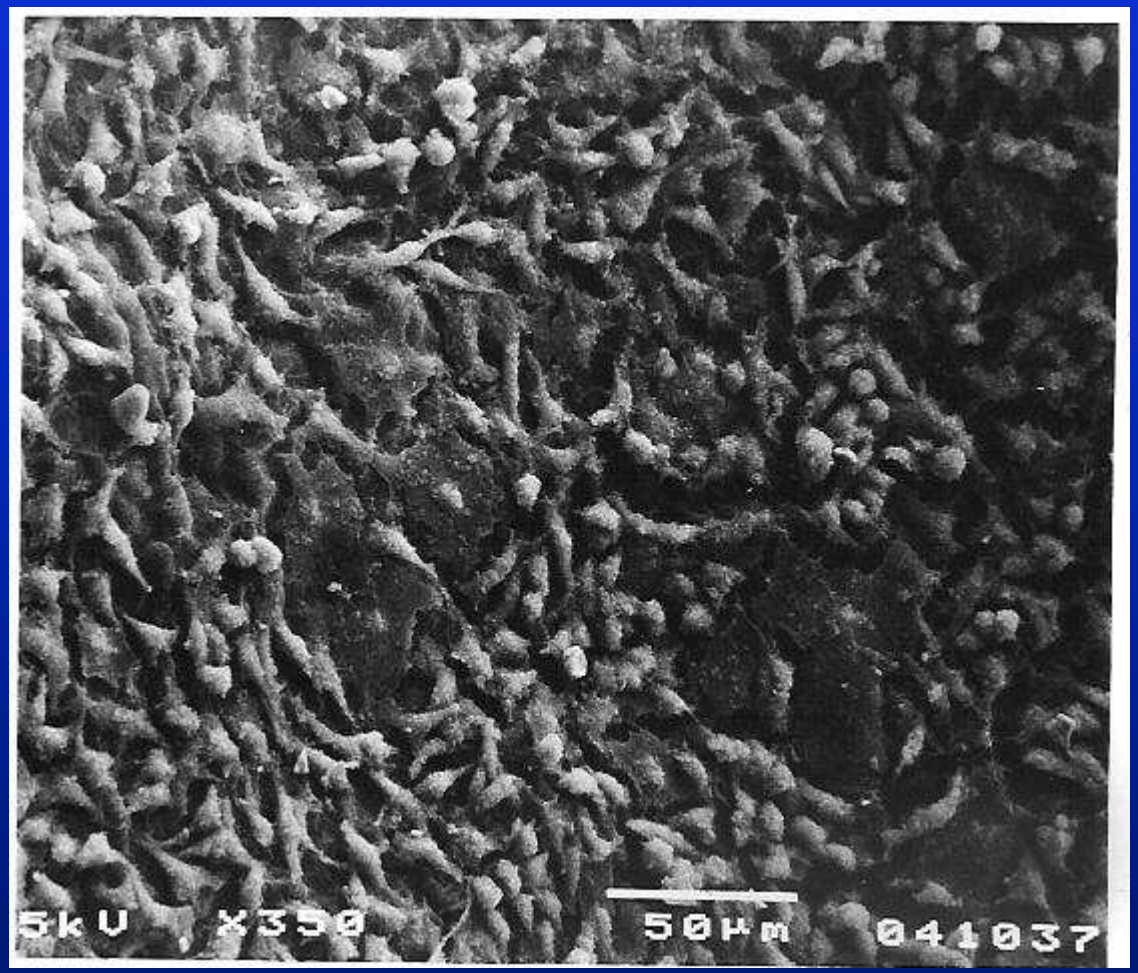
PEG-PDLLG-PEG and NHS were reacted in the presence of dicyclohexylcarbodiimide (DCC) and PEG-PDLLG-NHS was obtained.

Attachment of collagen onto PEG-PDLLG-PEG triblock copolymer:

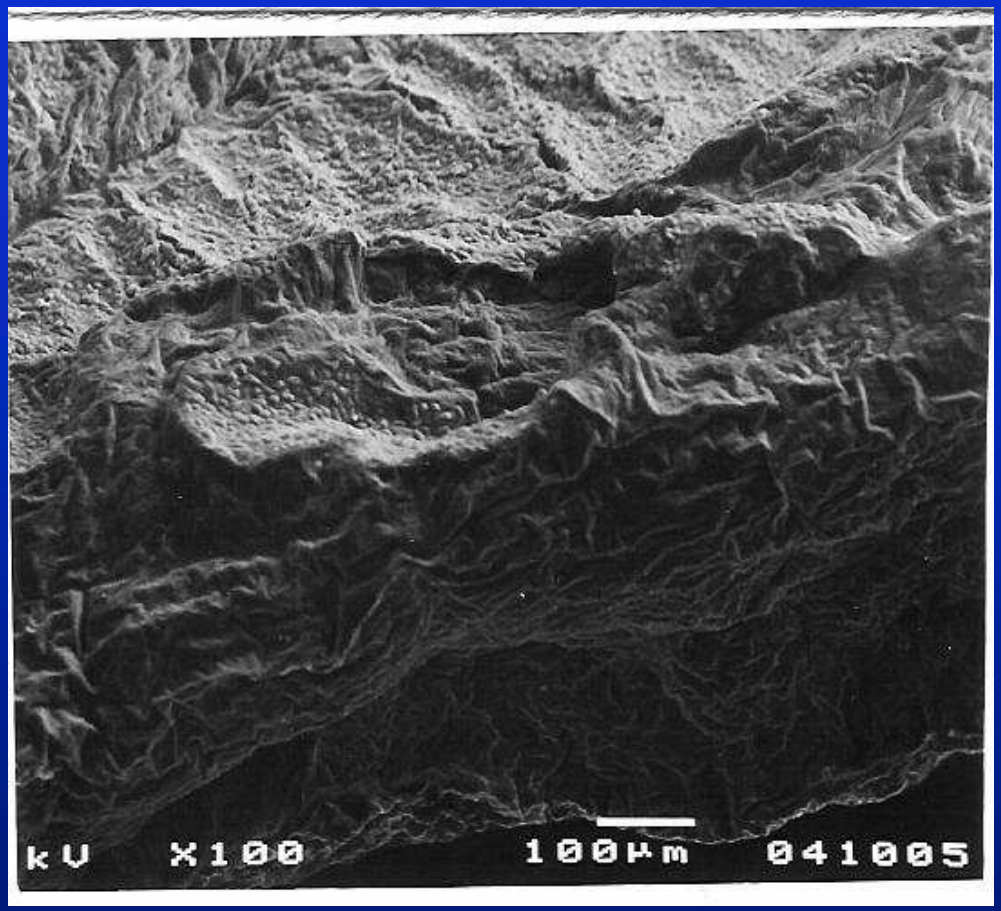
For modification of PEG-PDLLG-NHS copolymer, type I collagen from calf skin (Mw : 140 000 g/mol) was used.

The final product, PEG-PDLLG-Col, was used as a substrate for cell growth experiments.

Polymeric Samples	*M*w gmol^{-1}	*M*n gmol^{-1}
PEG-COOH	1 160	1 110
PDLLG	23 460	14 560
PEG-PDLLG-PEG	25 900	21 700
PEG-PDLLG-NHS	37 650	25 240

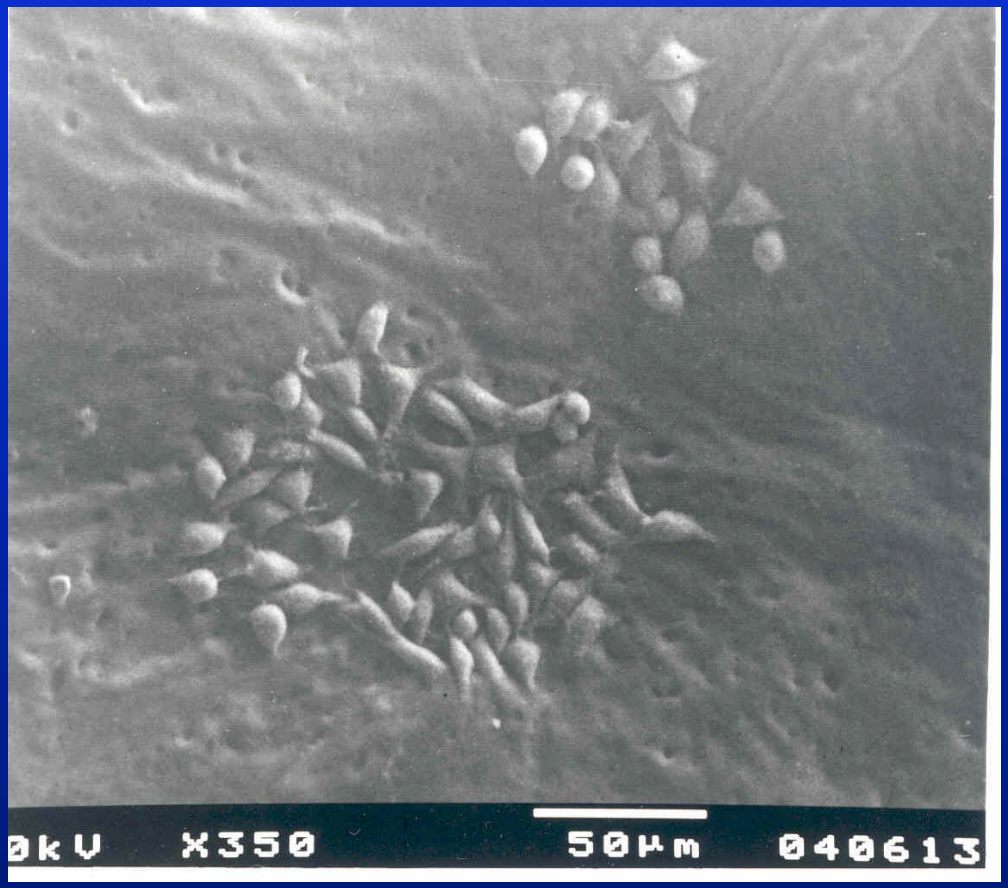


L929 cells on PDLLG



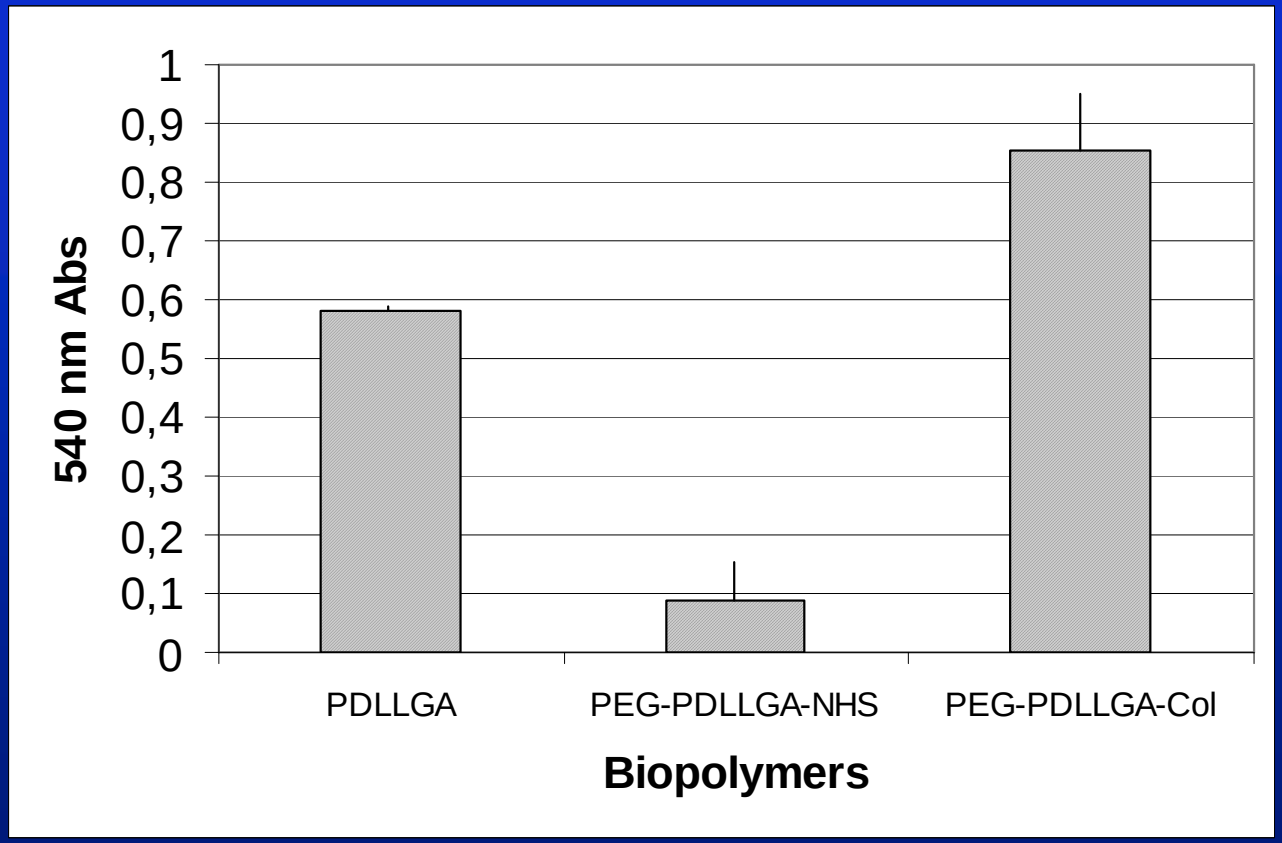
L929 cells on PEG-PDLLG-Col

SEM photos: Feriha Şirvancı, Marmara Univ.



L929 cells on PEG-PDLLG-NHS

SEM photos: Feriha Şirvancı, Marmara Univ.



Cell viability assay by neutral red uptake. 150,000 L929 cells were seeded per 22 mm diameter polymer films and grown for 72 hours in a CO_2 incubator.

Conclusion

A triblock copolymer of PEG-PDLLG-PEG was synthesized and characterized.

A suspension of this PEG-PDLLG-Col polymer was poured onto PDLLG films formed in Teflon molds and a new polymeric film was prepared. Similarly, films of PDLLG and PEG-PDLLG-NHS were made.

The biocompatibility of PEG-PDLLG-Col was compared with the latter materials by assessing the attachment and growth of L929 mouse fibroblasts on these films in vitro.

Both SEM analysis and neutral red uptake assay following a 72-hour incubation indicated that collagen modification significantly increased the number of cells on PDLLG.

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THANK YOU FOR LISTENING

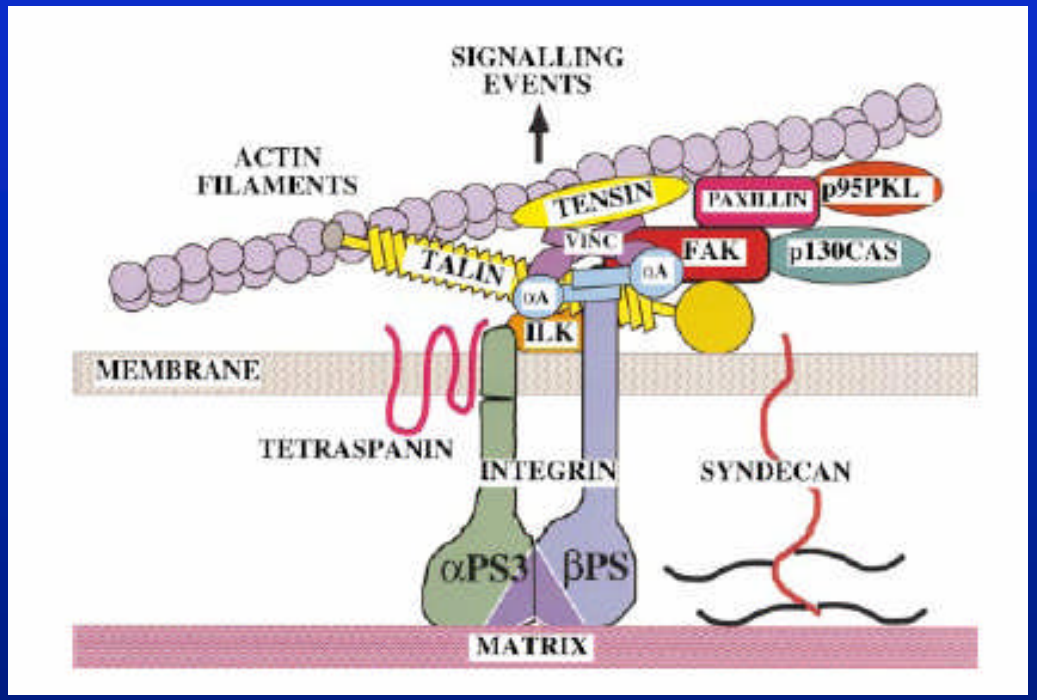
SIGNALLING
EVENTS
ACTIN
FILAMENTS
TENSIN
PAXILLIN
p95PKL
VINC
FAK
p130CAS
TALIN
αA
αA
ILK
MEMBRANE
TETRASPANIN
INTEGRIN
SYNDECAN
αPS3
βPS
MATRIX

TABLE 22-3 Major Collagen Molecules

Type	Molecule Composition	Structural Features	Representative Tissues
Fibrillar Collagens			
I	$[\alpha1(I)]_2[\alpha2(I)]$	300-nm-long fibrils	Skin, tendon, bone, ligaments, dentin, interstitial tissues
II	$[\alpha1(II)]_3$	300-nm-long fibrils	Cartilage, vitreous humor
III	$[\alpha1(III)]_3$	300-nm-long fibrils; often with type I	Skin, muscle, blood vessels
V	$[\alpha1(V)]_3$	390-nm-long fibrils with globular N-terminal domain; often with type I	Similar to type I; also cell cultures, fetal tissues
Fibril-Associated Collagens			
VI	$[\alpha1(VI)][\alpha2(VI)]$	Lateral association with type I; periodic globular domains	Most interstitial tissues
IX	$[\alpha1(IX)][\alpha2(IX)][\alpha3(IX)]$	Lateral association with type II; N-terminal globular domain; bound glycosaminoglycan	Cartilage, vitreous humor;
Sheet-Forming Collagens			
IV	$[\alpha1(IV)]_2[\alpha2(IV)]$	Two-dimensional network	All basal laminaes

source: K. Kuhn, 1987, in R. Mayne and R. Burgeson, eds., *Structure and Function of Collagen Types*, Academic Press, p. 2; M. van der Rest and R. Garrone, 1991, *FASEB J.* 5:2814.