UNIVERSITÁ DEGLI STUDI DI TERAMO

Corso di Laurea Magistrale in Biotecnologie Avanzate
AA 2022-2023

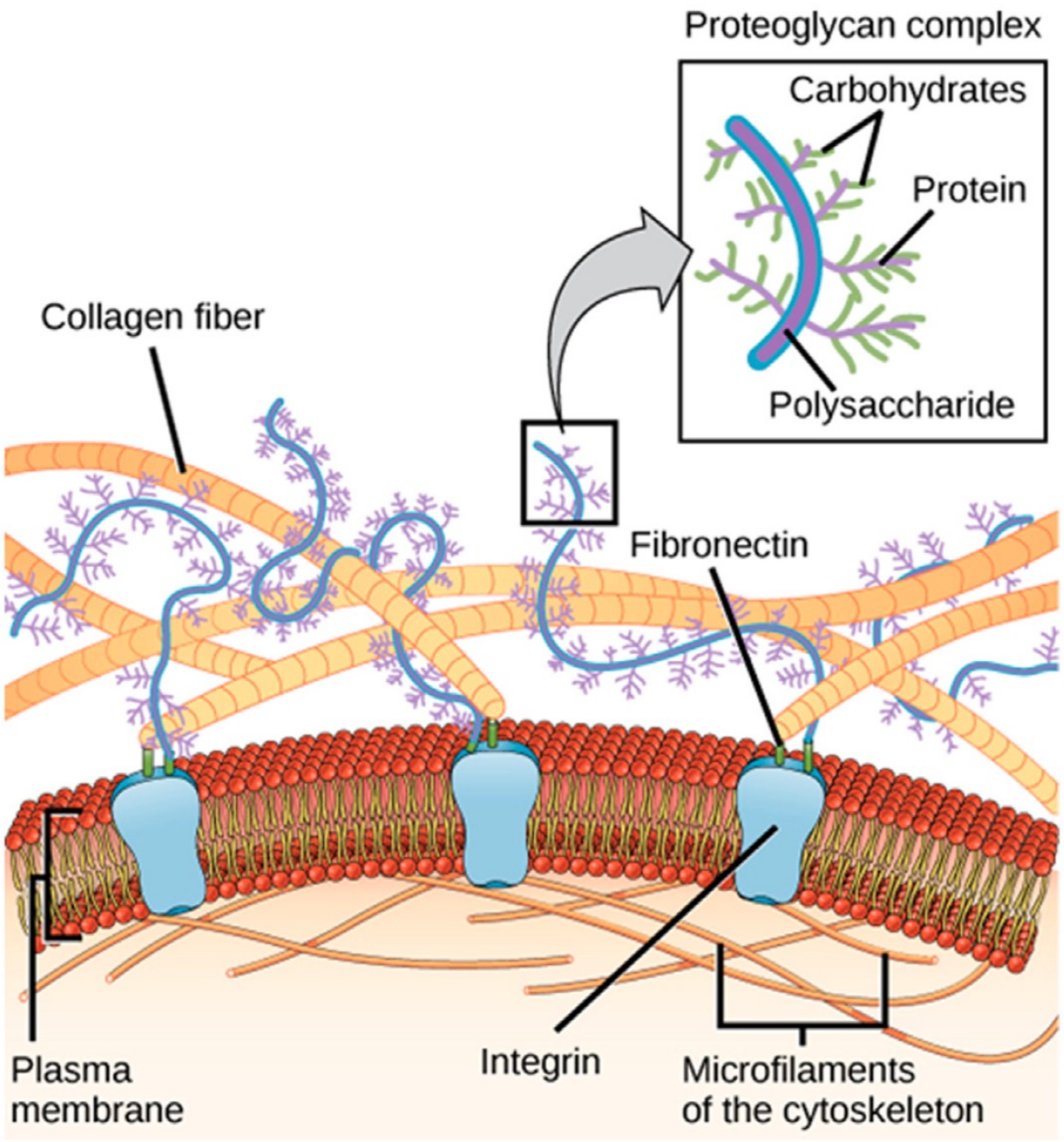
Functionalization Techniques of Medical Devices

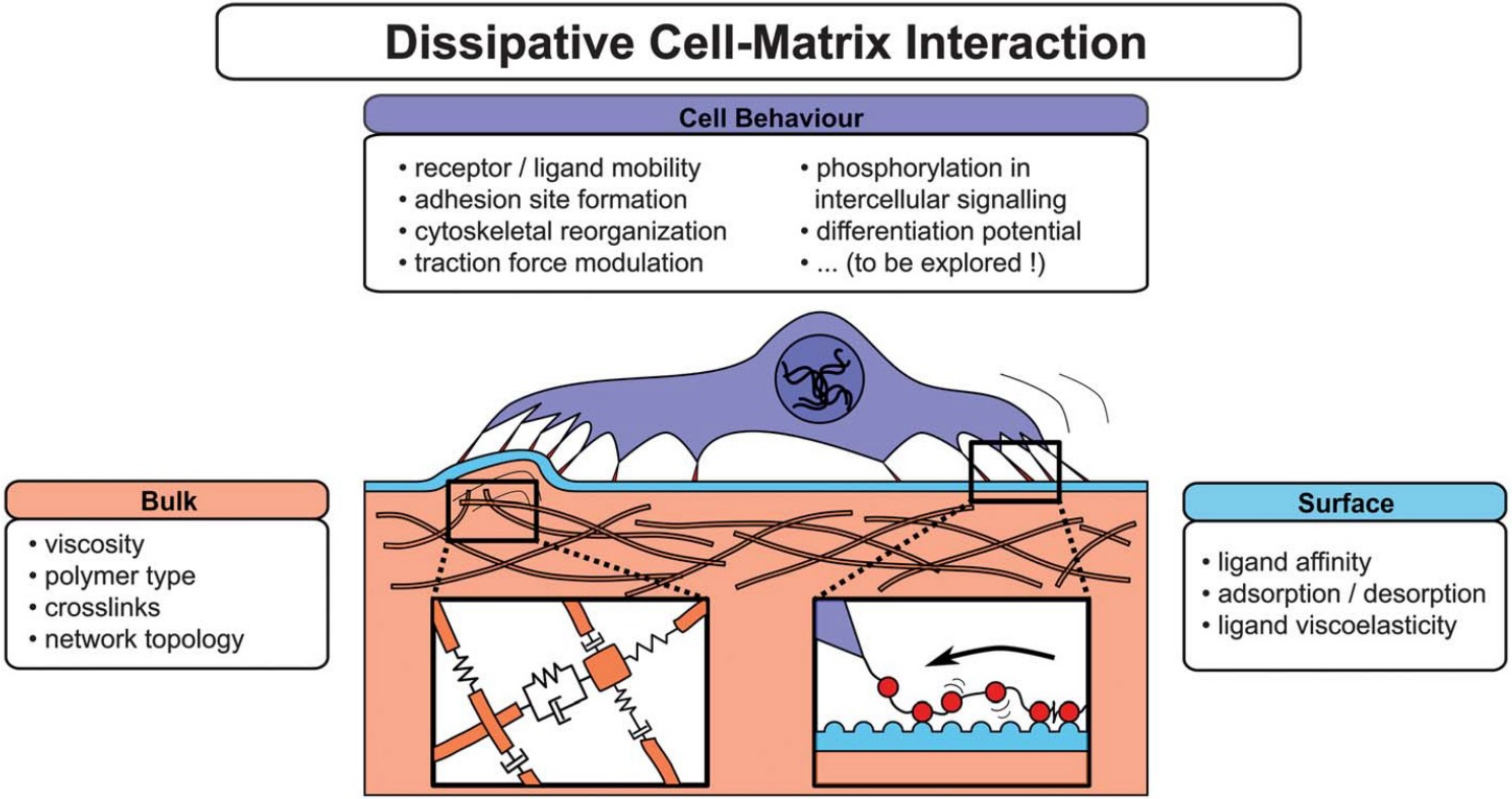
- Prepare scaffolds with biomimetic properties in respect to those of the **ECM of the tissue to be engineered**, including: biomimetic mechanical properties, chemical composition, and architecture.

- Main ECM proteins include **structural** and **cell adhesion proteins** able to interact with cell surface receptors.

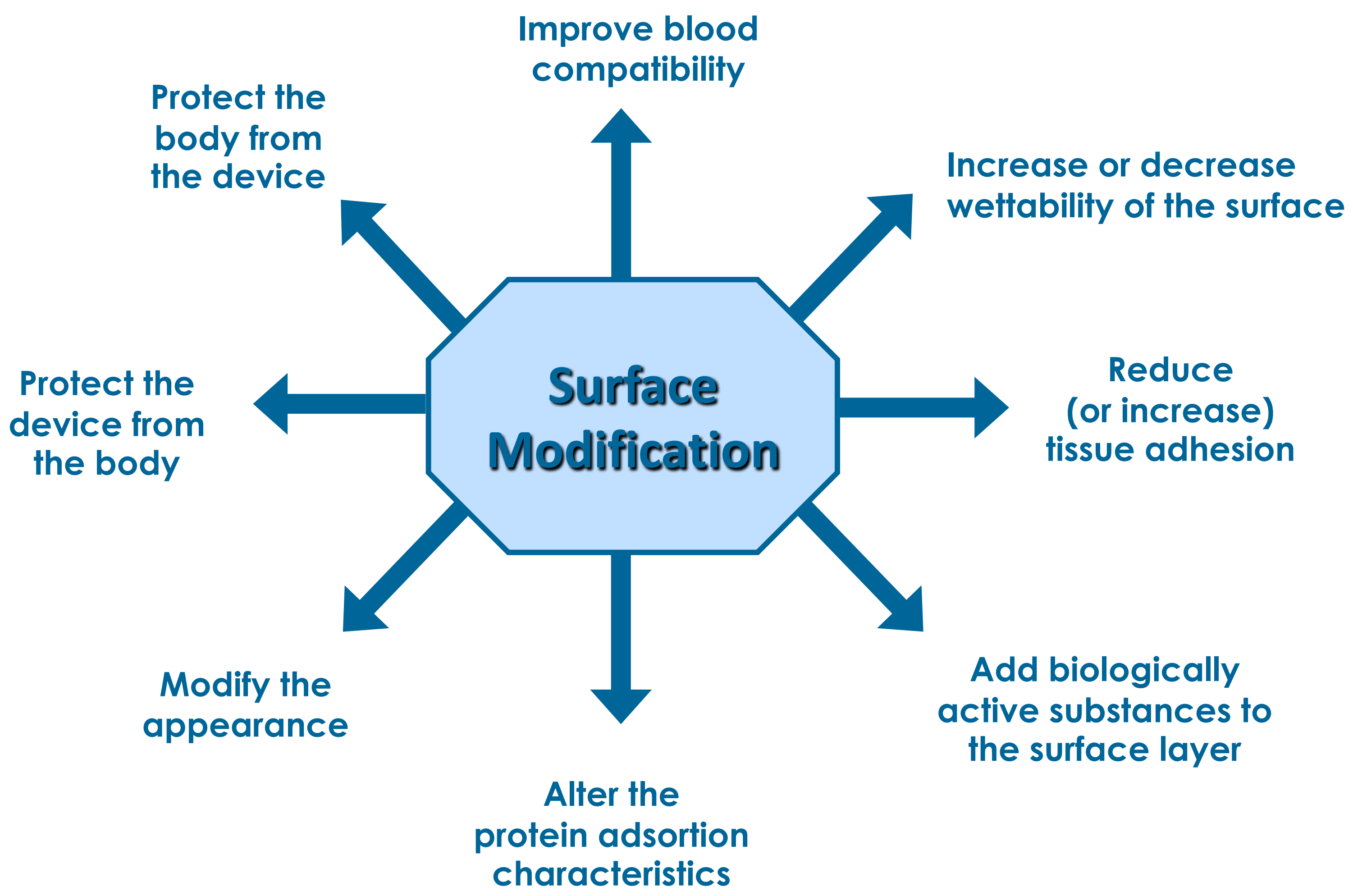
- **Glycosaminoglycans** and **proteoglycans** mainly <u>regulate the level of hydration of natural ECM, its permeability and the traffic and activity of soluble molecules secreted by cells.</u>

- Each ECM has its proper composition, architecture, and topography.





Müller et al. 2013, Soft Matter, DOI: 10.1039/c3sm50803j

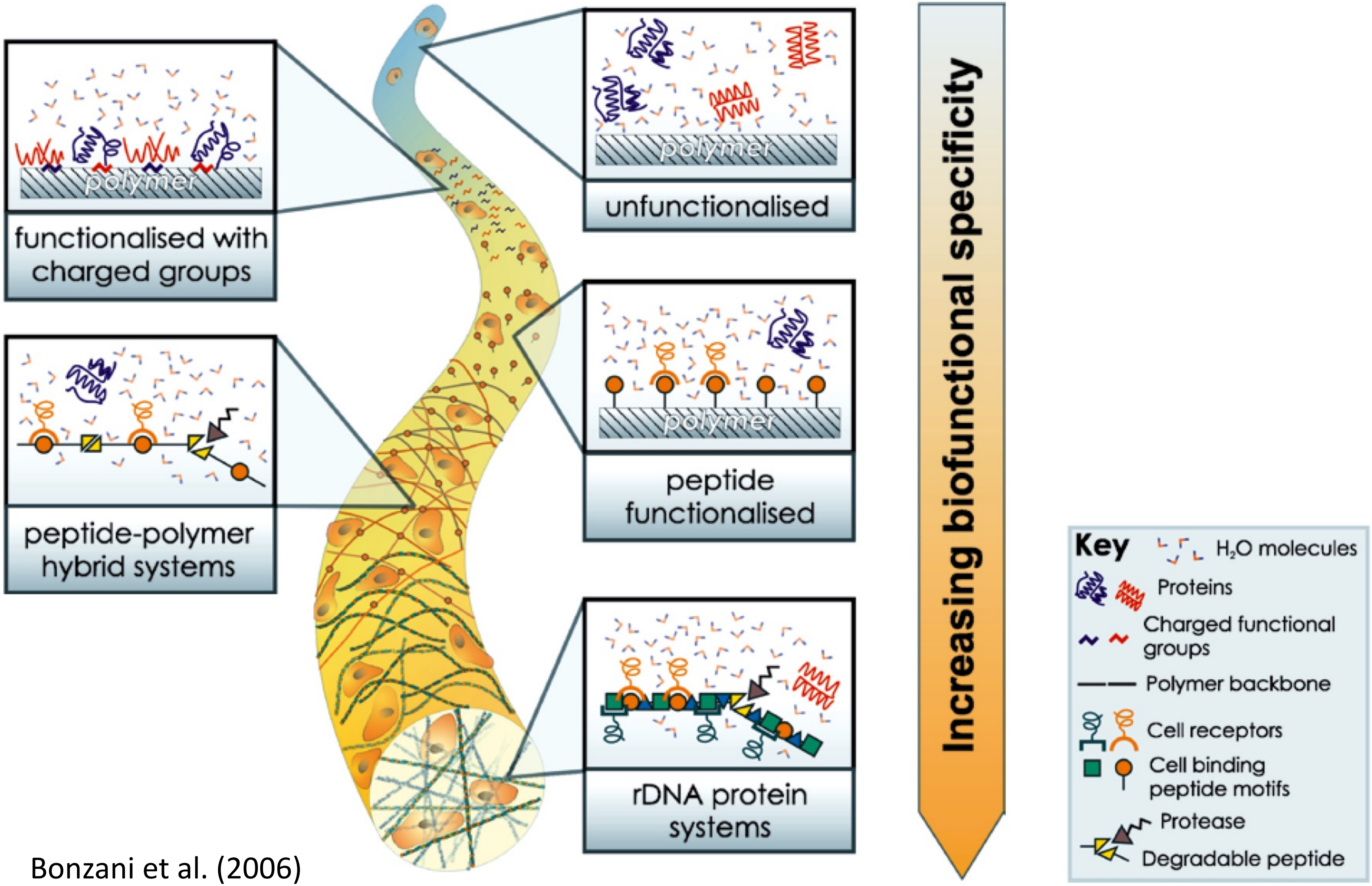

Improve blood compatibility
Protect the body from the device
Increase or decrease wettability of the surface
Surface Modification
Protect the device from the body
Reduce (or increase) tissue adhesion
Modify the appearance
Add biologically active substances to the surface layer
Alter the protein adsorption characteristics

PRO/CON

Polymers	Advantages	Disadvantages
Natural (proteins and polysaccharides)	- Biocompatible and bioactive - Biological origin	- Faster degradation rate - Poor mechanical properties - Risk of contamination - Batch-to-batch variability - High production cost
Synthetic (polyesters, PCL, PU, etc, ..)	- High mechanical properties - Shape stability in physiological media - Tailored degradation rate - Low production cost - Low immune response	- Lack of cell recognition moieties to induce cell adhesion by integrin receptors - Risk of biodegradation side effects

It is crucial to introduce functional groups on the surface of the scaffold that will function as cell recognition sites or may act as focal points for additional modification with bioactive molecules

Tallawi et al., 2015, Interface 12: 20150254.
http://dx.doi.org/10.1098/rsif.2015.0254



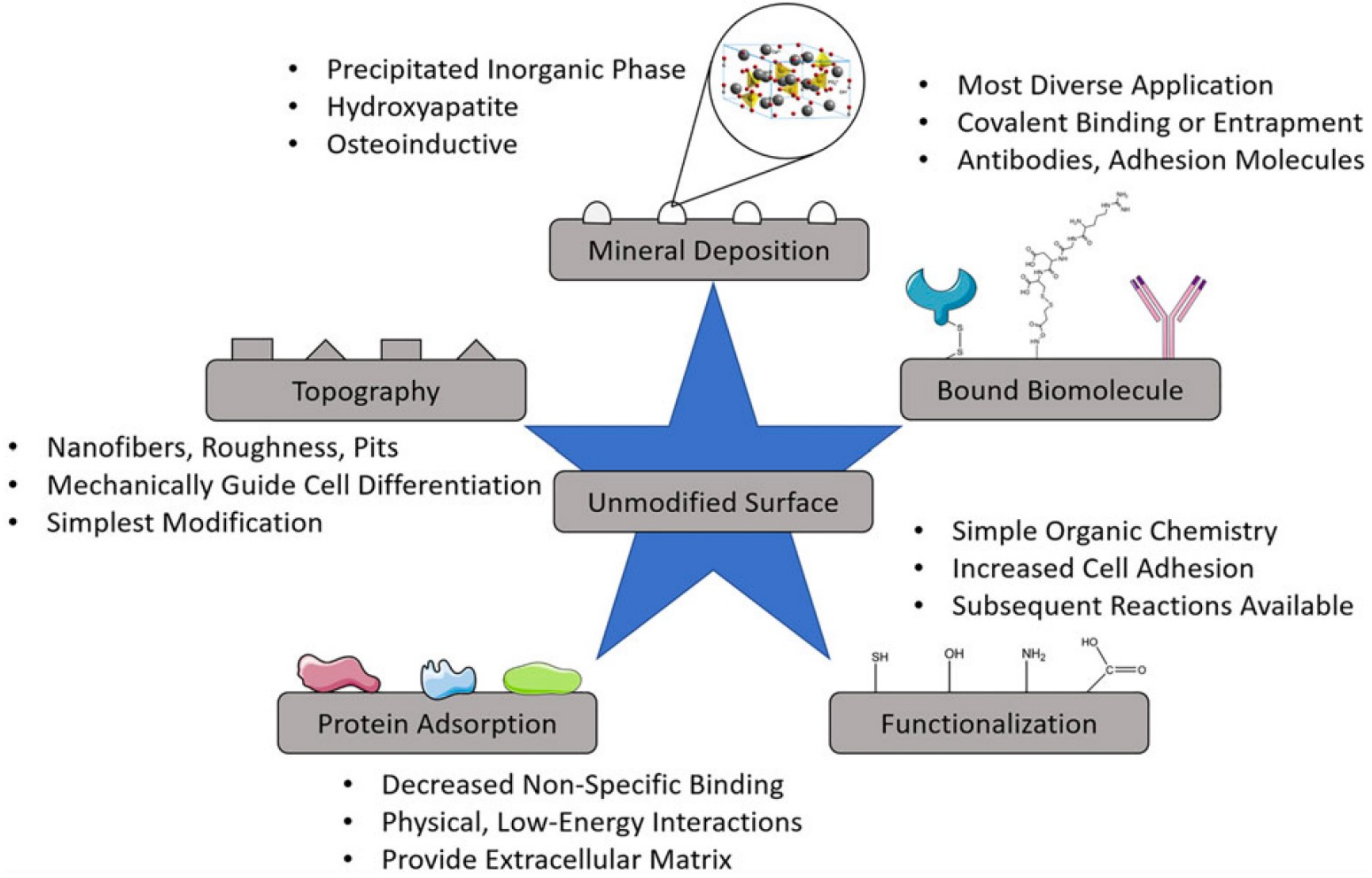
Bonzani et al. (2006)

Functionalization approaches

Bulk functionalization: by blending natural and synthetic polymers or by the synthesis of copolymers containing blocks based on synthetic and natural polymers.

Surface functionalization: with natural polymers or their bioactive fragments (e.g., peptides) of synthetic polymers substrates.

Carmagnola et al., Functional 3D Tissue Engineering Scaffolds

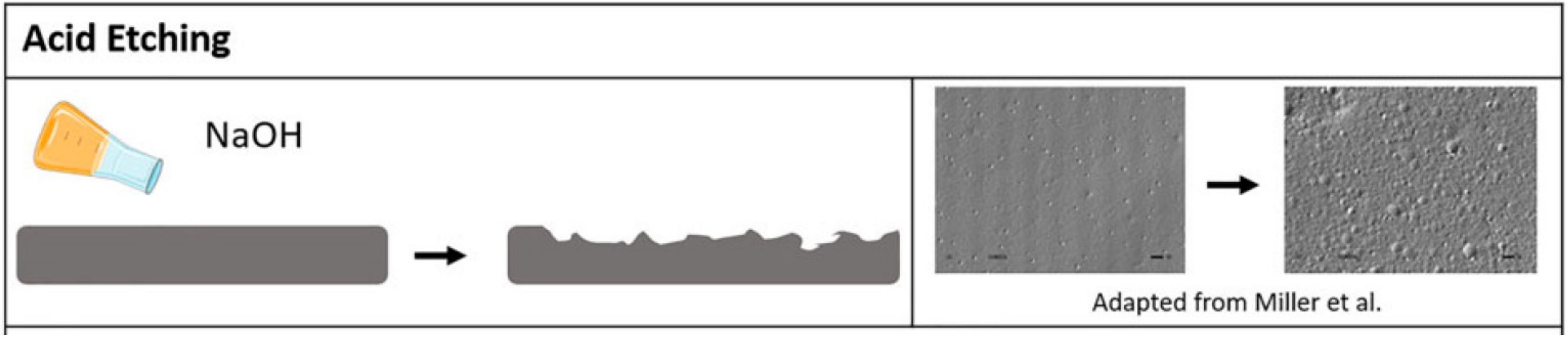


Richbourg et al., J Tissue Eng Regen Med. 2019;13:1275–1293.

Surface Functionalization Methods

- Pre-functionalization strategies
- Non-Covalent functionalization with bioactive molecules
- Covalent functionalization with bioactive molecules

TOPOGRAPHICAL MODIFICATION



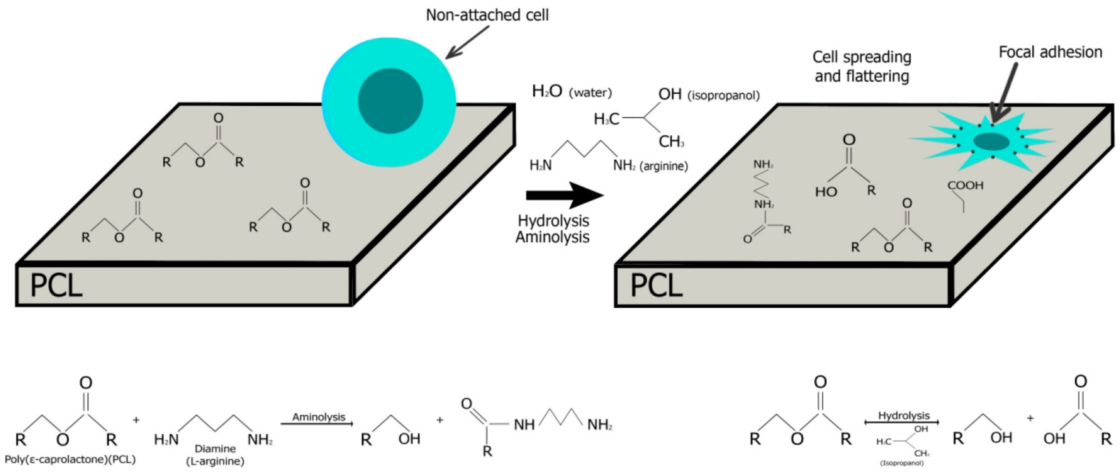
Aminolysis

Hydrolysis

Can be performed on polyester scaffolds

diamine solution

acidic or basic solution



Nashchekina, Int. J. Mol. Sci. 2020, 21, 6989; doi:10.3390/ijms21196989

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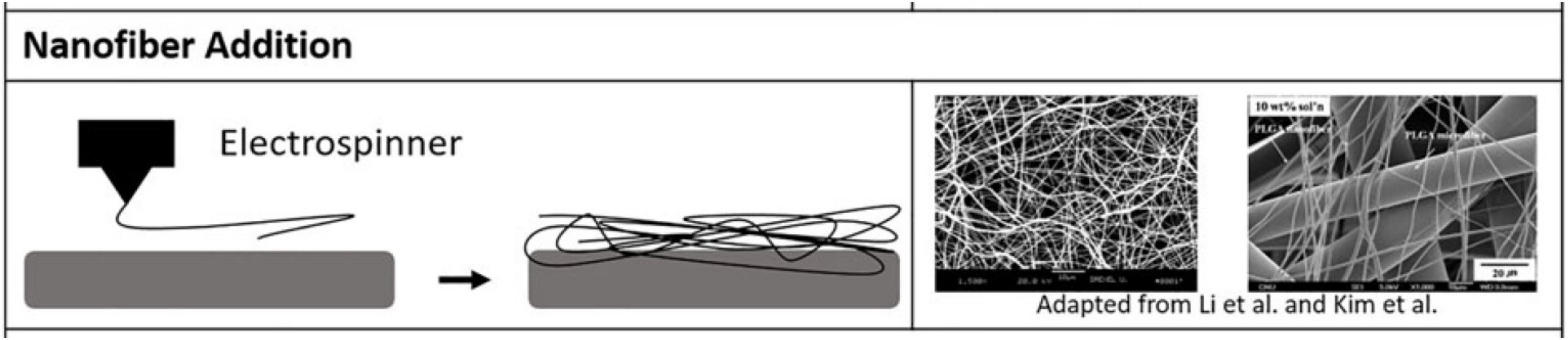
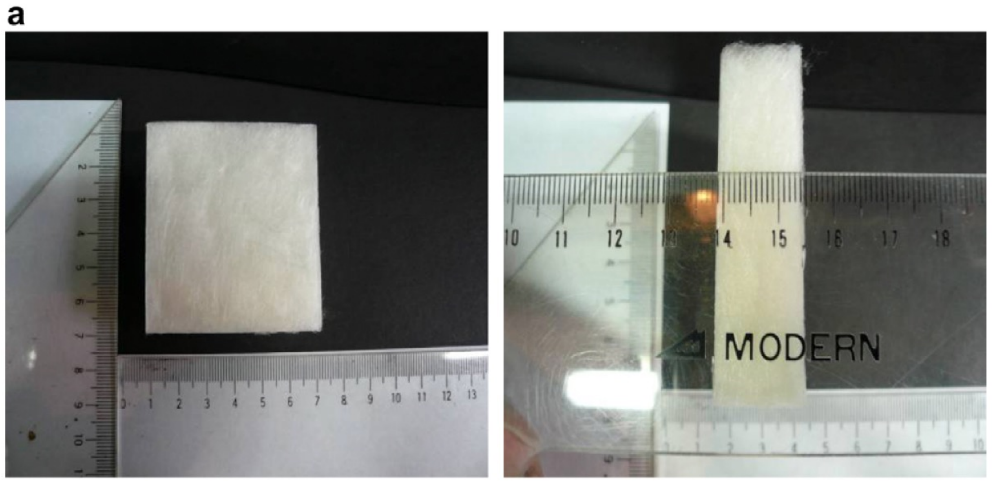
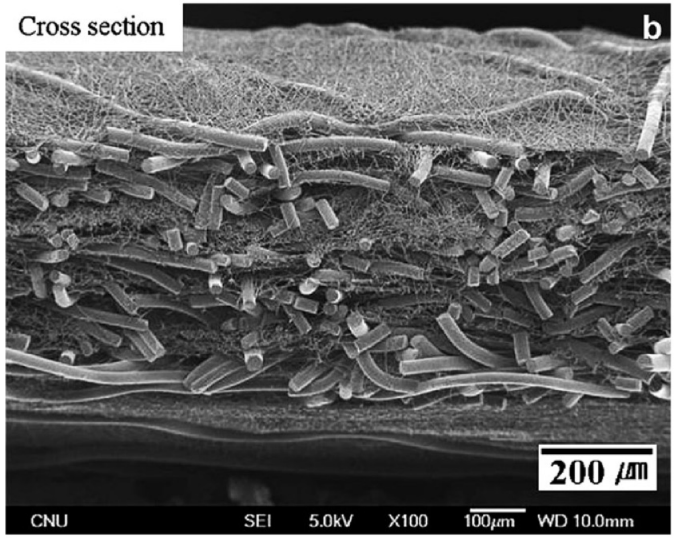
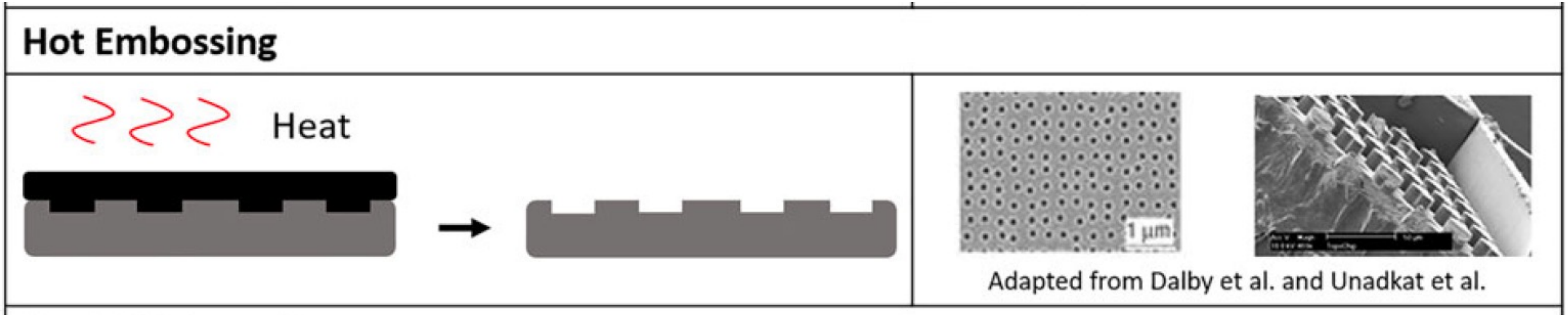


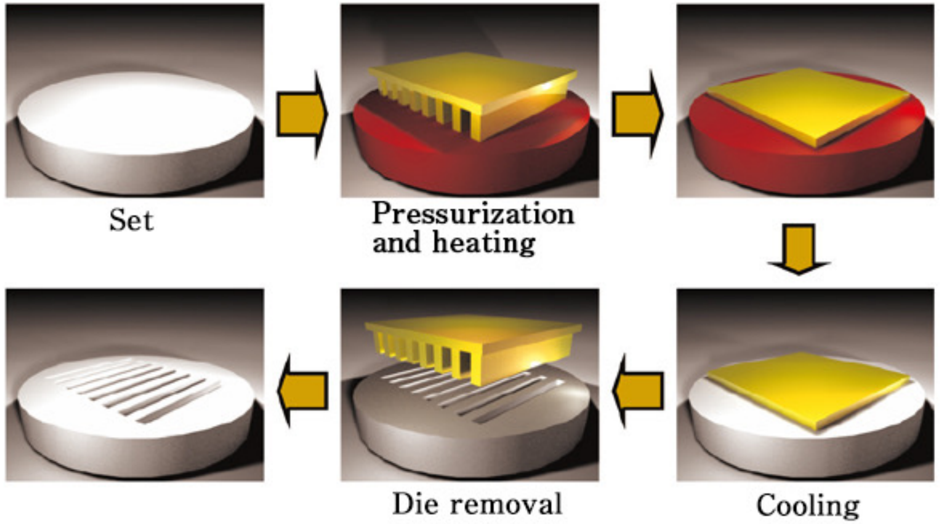


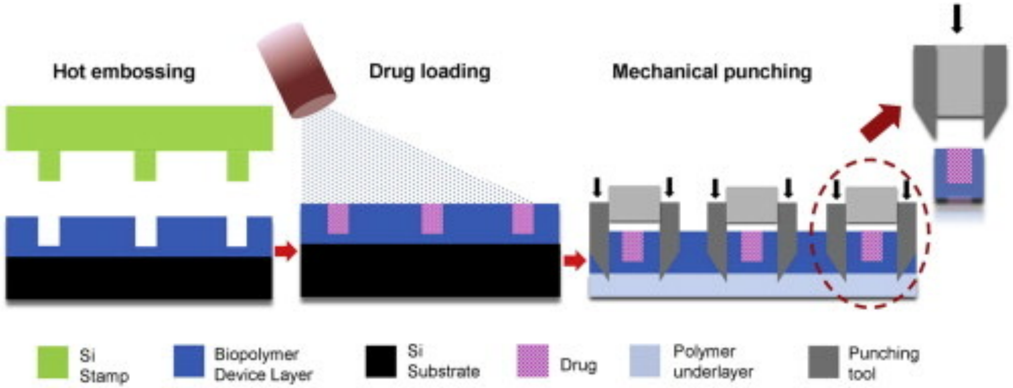


S.J. Kim et al. / Polymer 51 (2010) 1320–1327

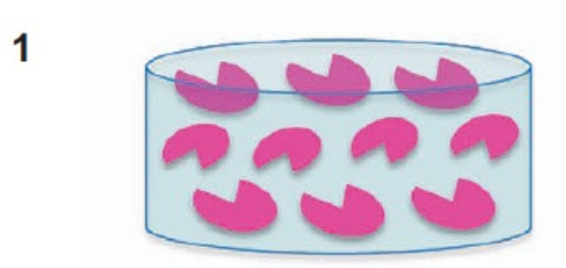
TOPOGRAPHICAL MODIFICATION



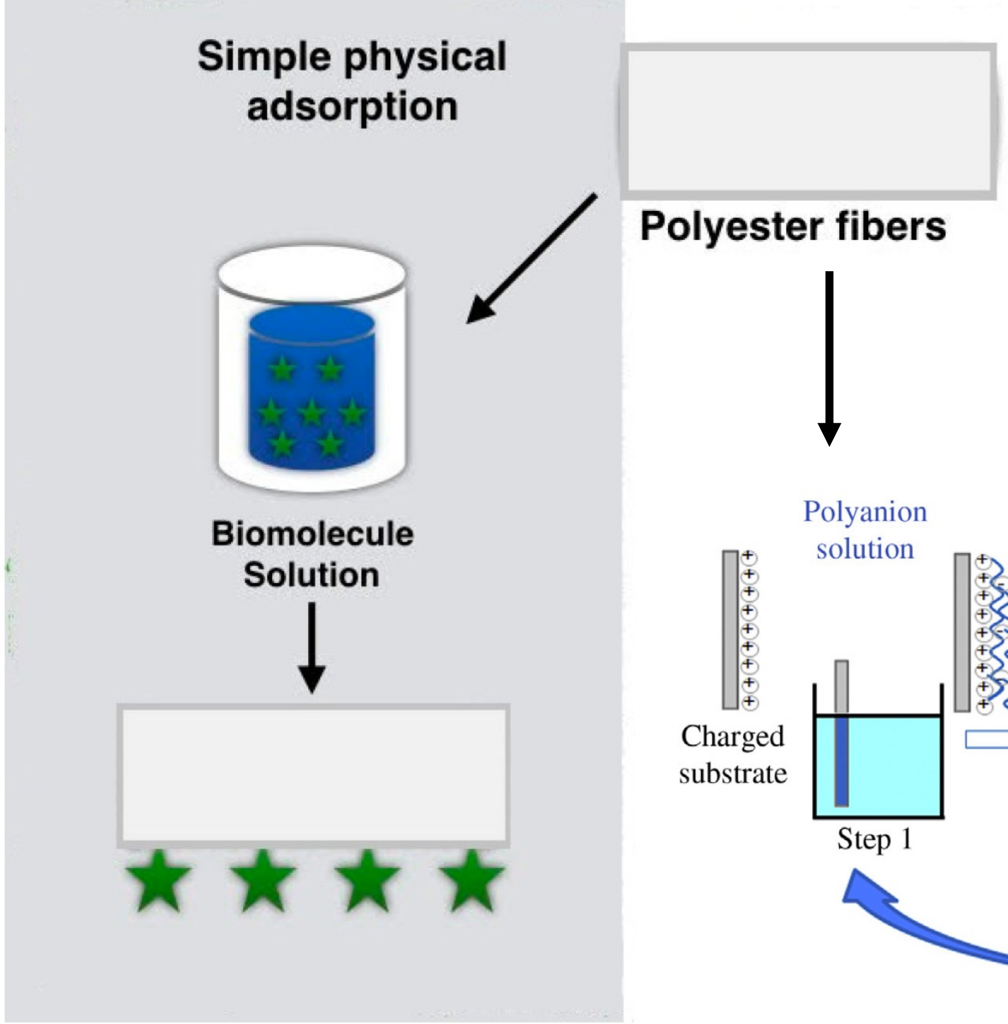




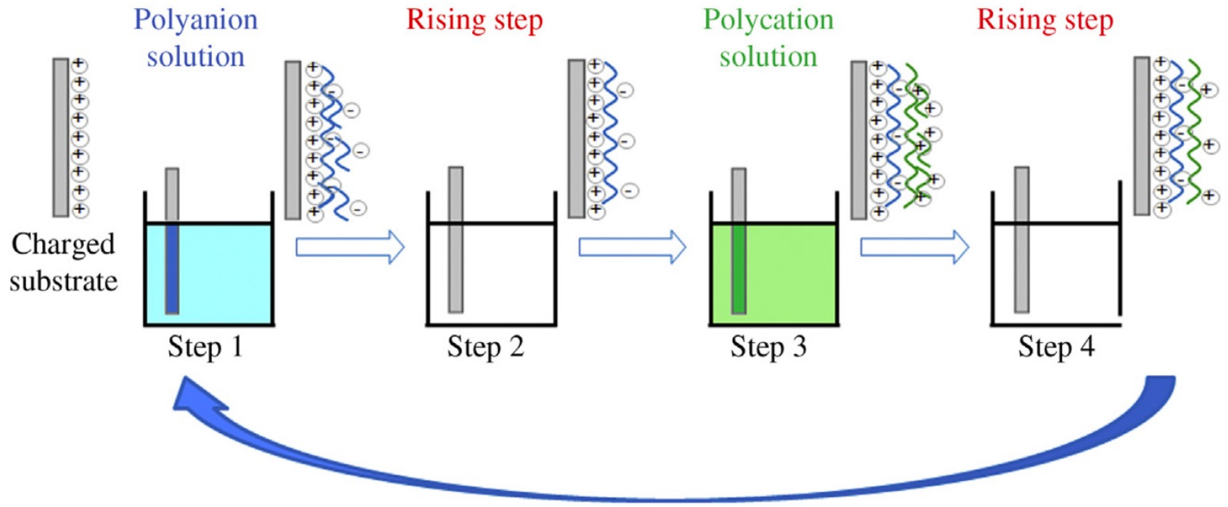
Non-Covalent functionalization with bioactive molecules



Physical encapsulation of GFs



Layer-by-Layer (LbL)

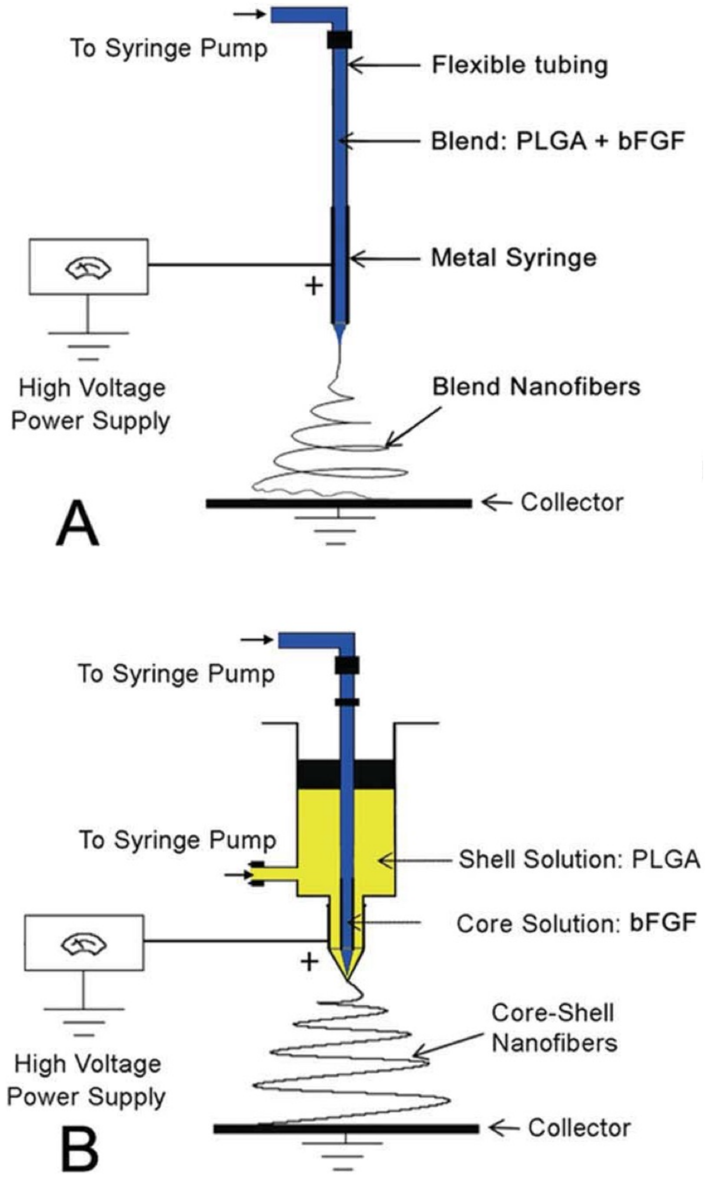


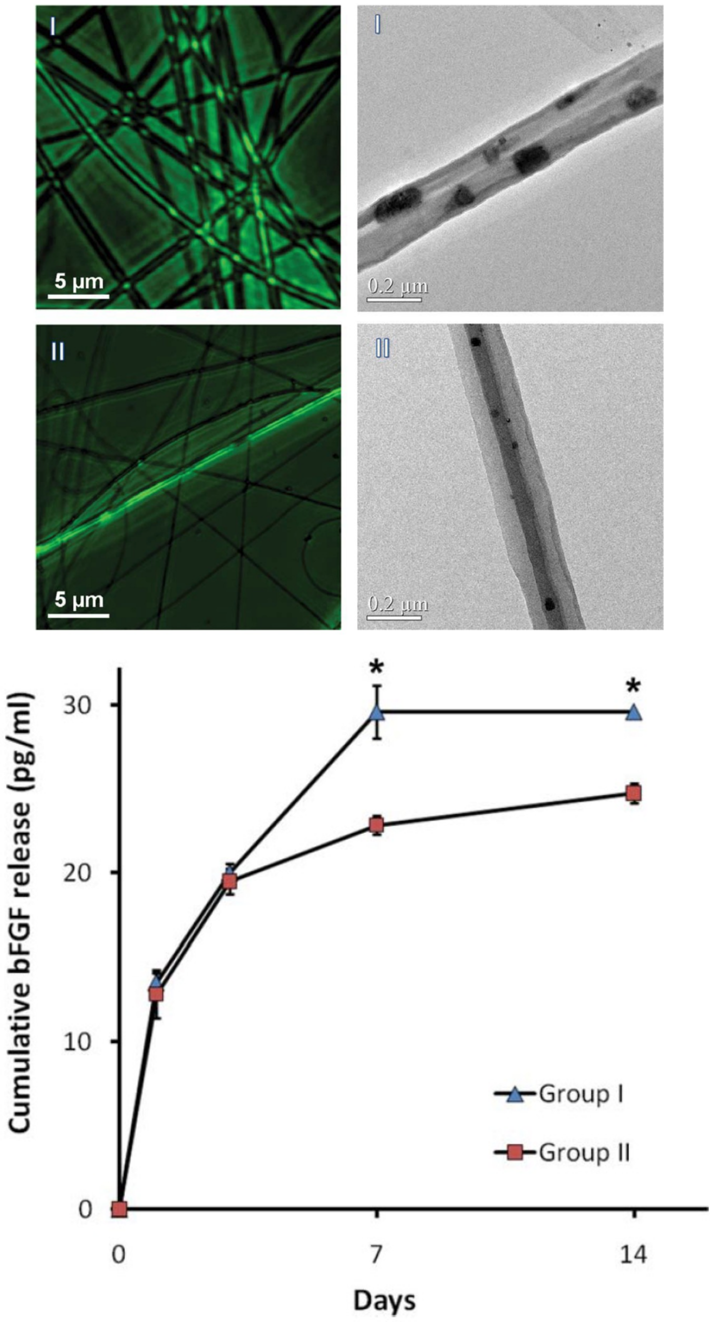
Niemczyk-Soczynska et al., Polymers 2020, 12, 2636

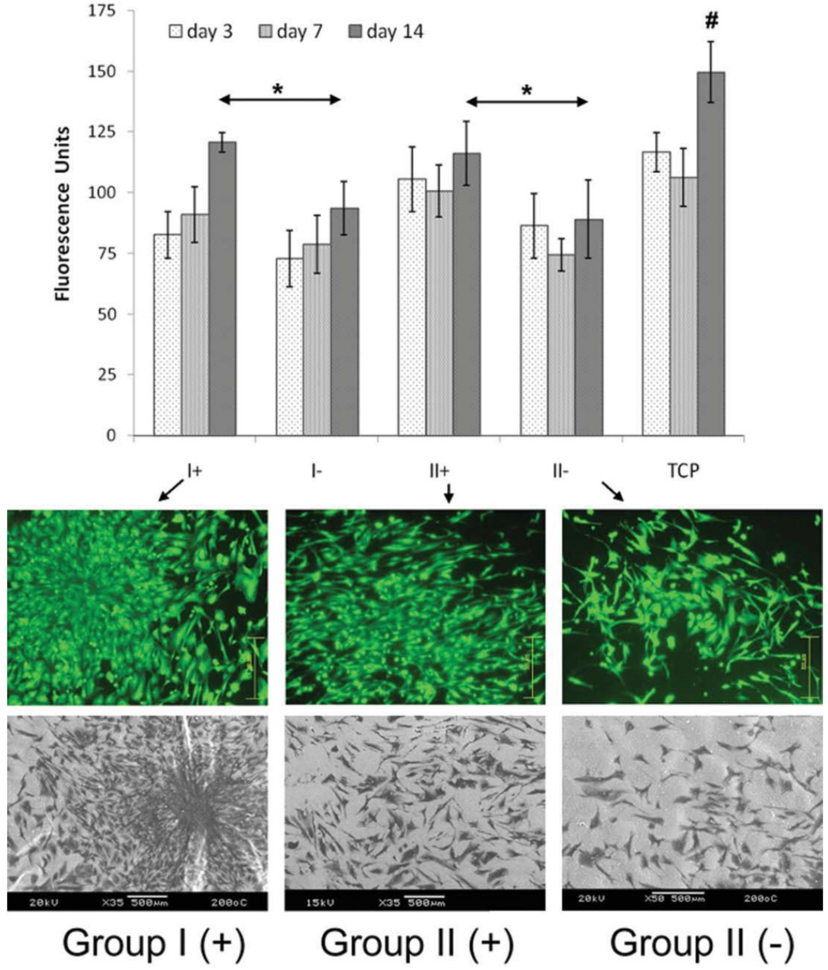
Carmagnola et al., 2018, Functional 3D Tissue Engineering Scaffolds

Physical Method	Mechanism	Advantages	Disadvantages
Simple physical adsorption	Weak physical interactions such as hydrophobic interactions, hydrogen bonds, van der Waals interactions [24,26]	• does not change bulk properties of the polymer [93] • protects biomolecules from challenging environment • simple, universal	• might change fibers morphology, for instance increases fibers thickness or clogs the pores [85] • impermanent [24]
LBL	Electrostatic interactions as an effect of alternate embedding of oppositely charged substances [26]	• does not change the bulk properties of polymer • protects biomolecules from a challenging environment [104] • simple, universal [26]	• only charged substances might be used [98,106] • modified surface needs to be charged, or previously pre-treated to deposit charge on the surface [97]

Niemczyk-Soczynska et al., Polymers 2020, 12, 2636

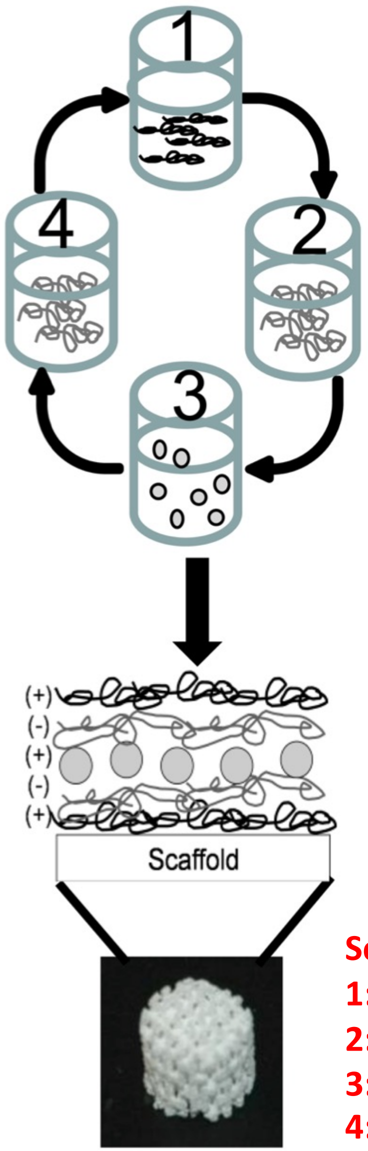


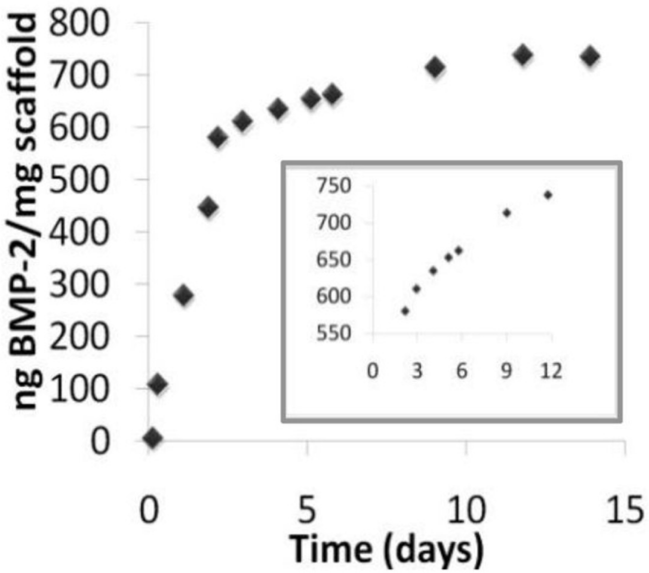


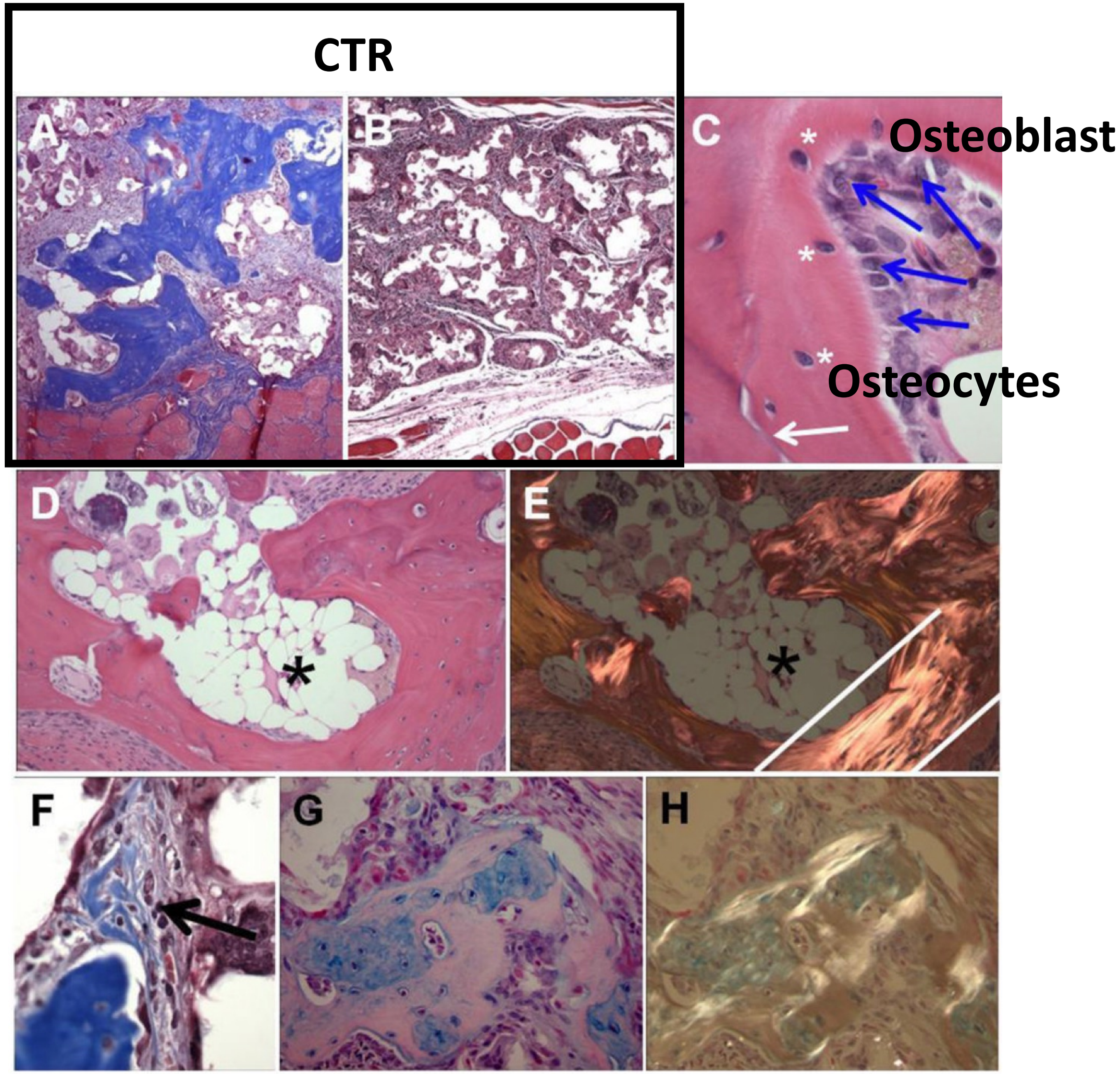


Sahoo et al., J Biomed Mater Res A. 2010 Jun 15;93(4):1539-50.
doi: 10.1002/jbm.a.32645.

Layer-by-Layer Self Assembly



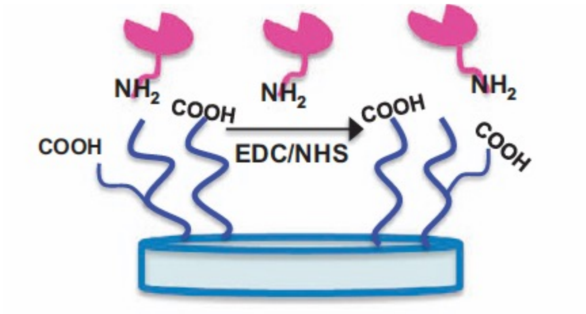




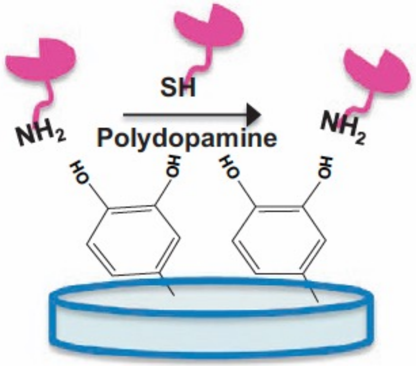
Scaffold: PCL/Tricalcium phosphate
1: Poly amino ester (+)
2: Chrondroitin sulfate (-)
3: BMP-2 (+)
4: Chondroitin sulfate (-)

Macdonald et al., Biomaterials. 2011 February ; 32(5): 1446–1453.
doi:10.1016/j.biomaterials.2010.10.052.

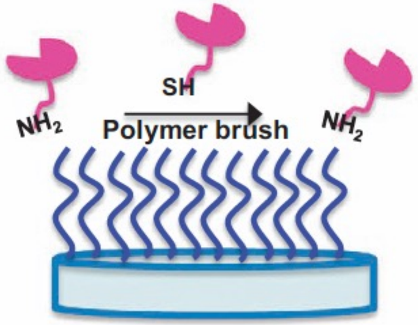
Covalent functionalization with bioactive molecules



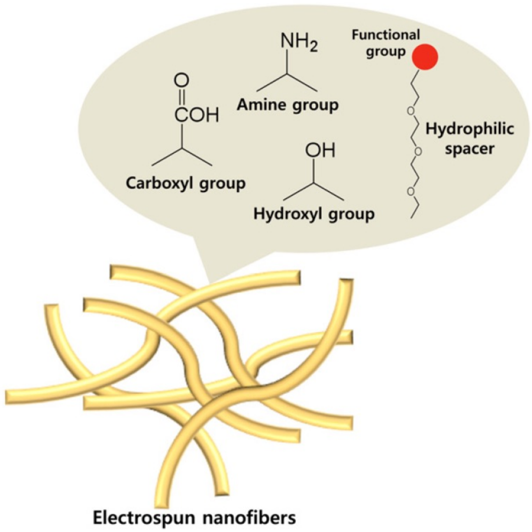
Carbodiimide coupling immobilization (EDC)



Mussel-inspired bioconjugations (PDA)



Other Chemical Coupling



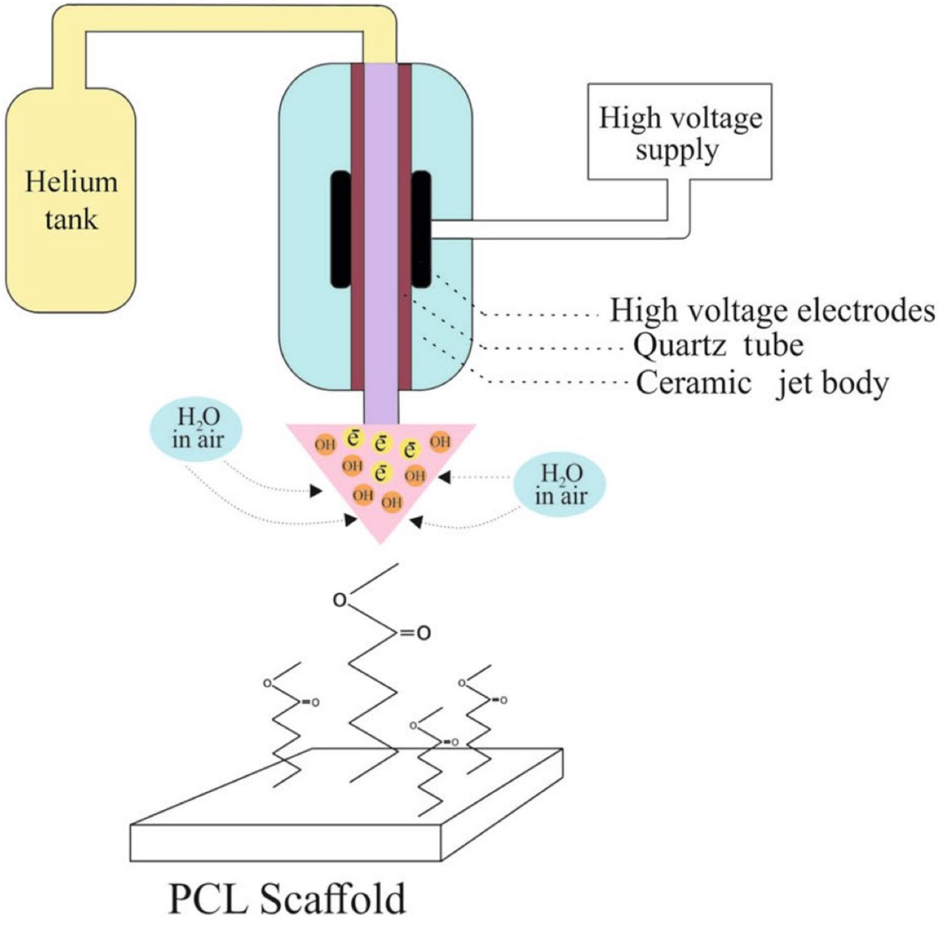
Surfaces pre-functionalized with amino groups can be grafted with amino-containing molecules by exploiting coupling reagents, such as glutaraldehyde or diethyleneglycol diglycidyl ether.

Primary amine and carboxylate groups were most extensively employed to immobilize bioactive molecules onto the surface of nanofibers.

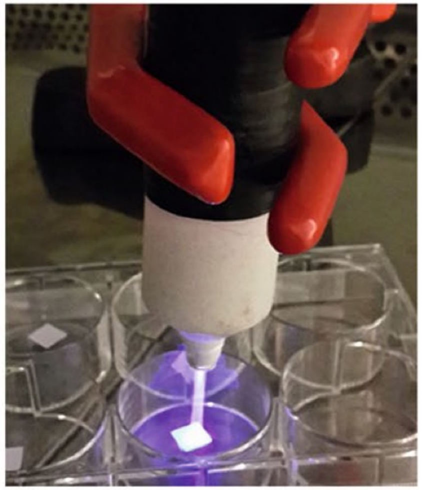
Upon activation of the carboxylic acid groups by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccimide (NHS), nanofibers were subsequently conjugated to primary amine groups of bioactive molecules.

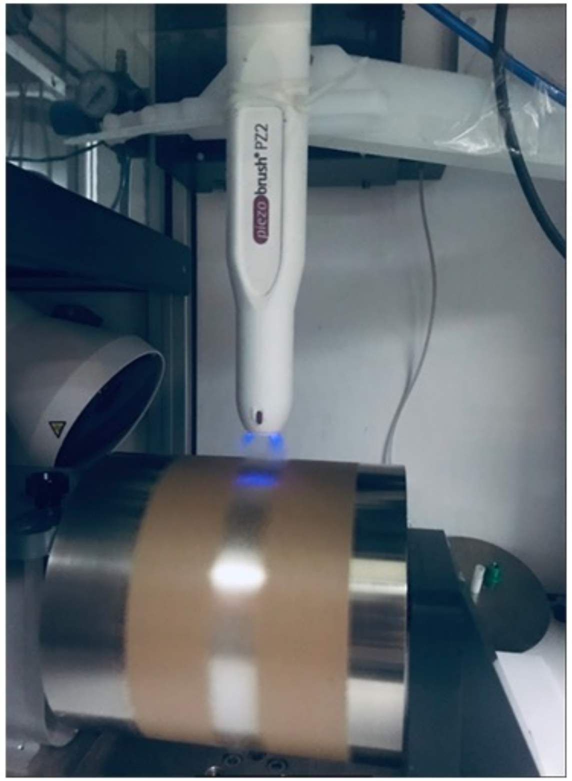
Carboxylic groups on the surface of polymeric nanofibers containing different amounts of polyacrylic acid were employed for conjugation with collagen.

Plasma Treatment

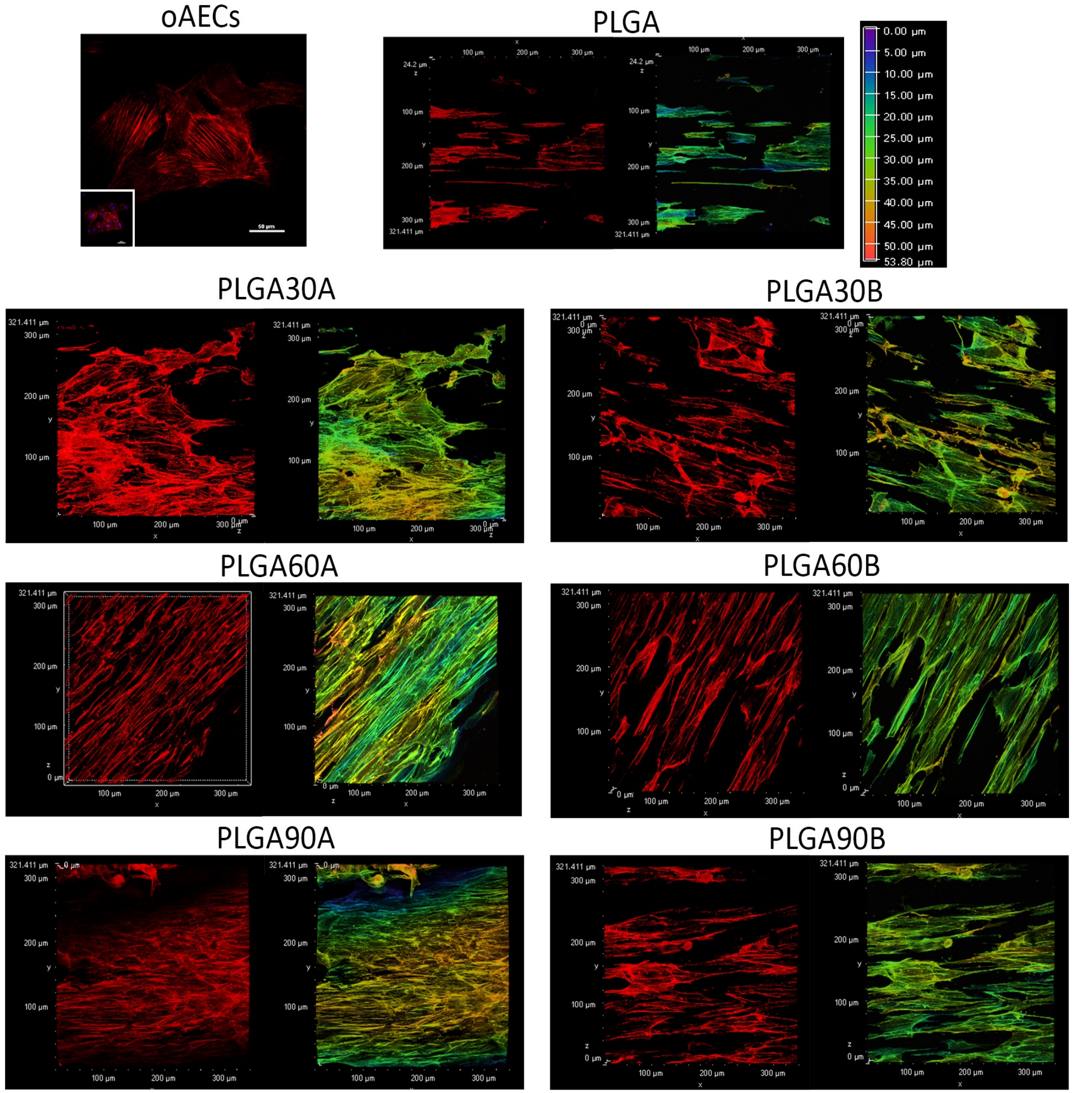


Meghdadi et al., Progress in Biomaterials (2019) 8:65–75



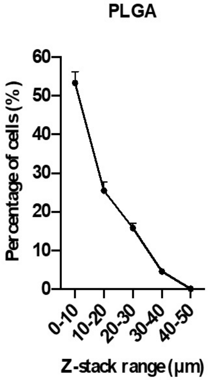


El Khatib et al., Molecules 2020, 25, 3176

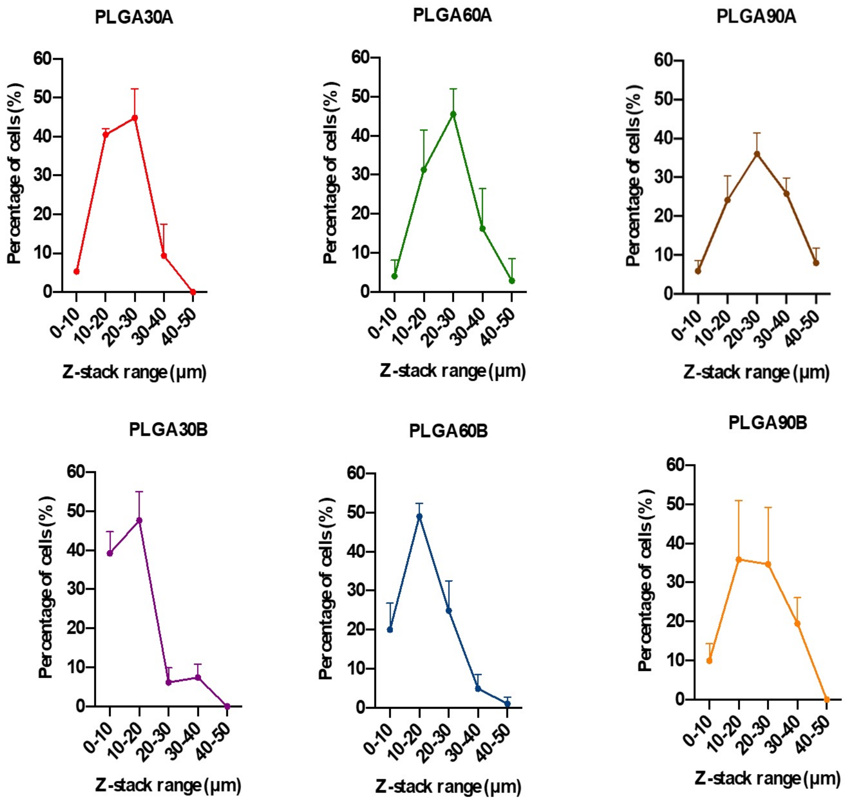


depth coded Maximum Intensity Projection (MaxIP).

Non-Treated

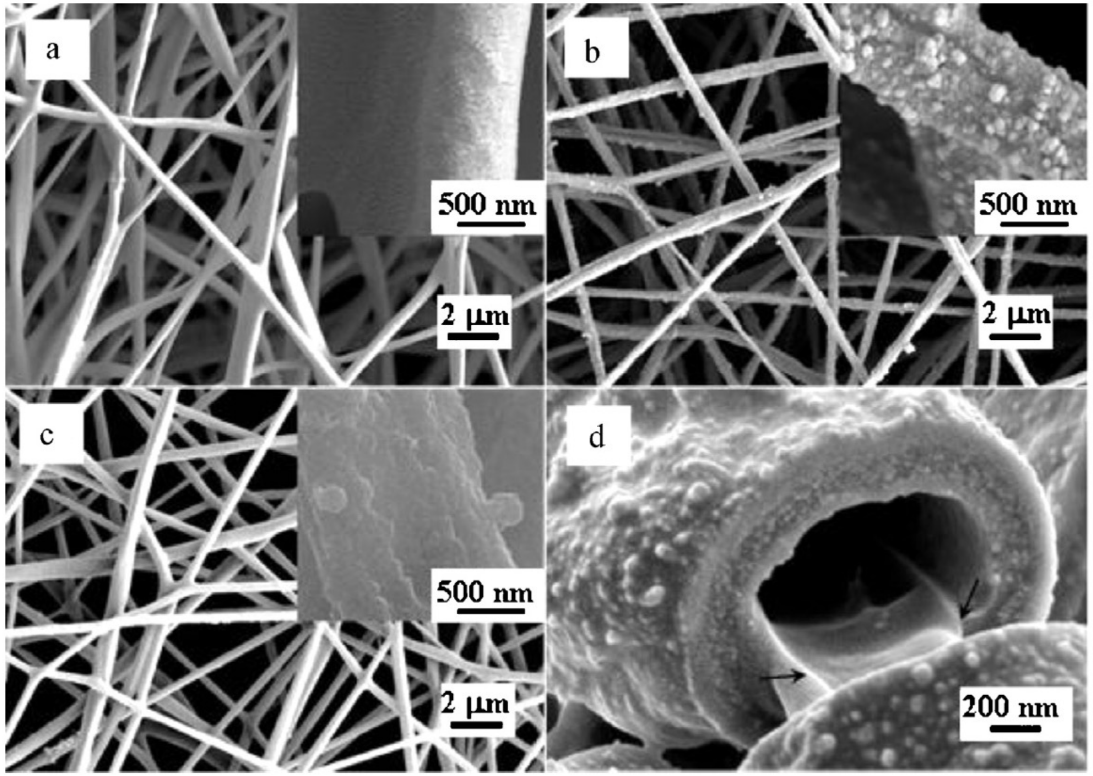


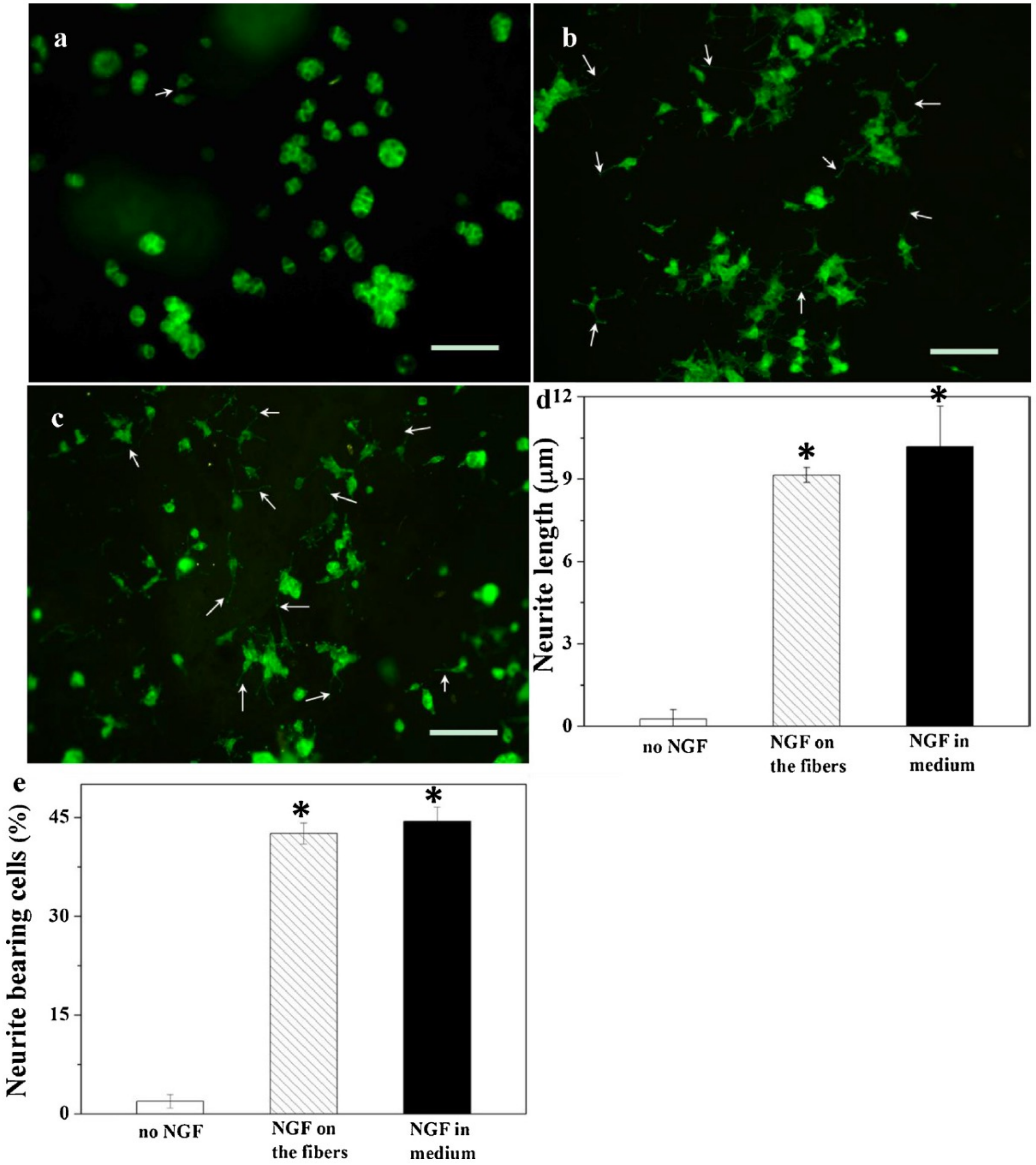
Treated with Cold Atmospheric Plasma



El Khatib et al., Molecules 2020, 25, 3176

Synthesis the conductive NGF-conjugated PPy–PLLA composite fibers byoxidation polymerization and ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDC) chemistry.





J. Zeng et al. / Colloids and Surfaces B: Biointerfaces *110 (2013) 450– 457*

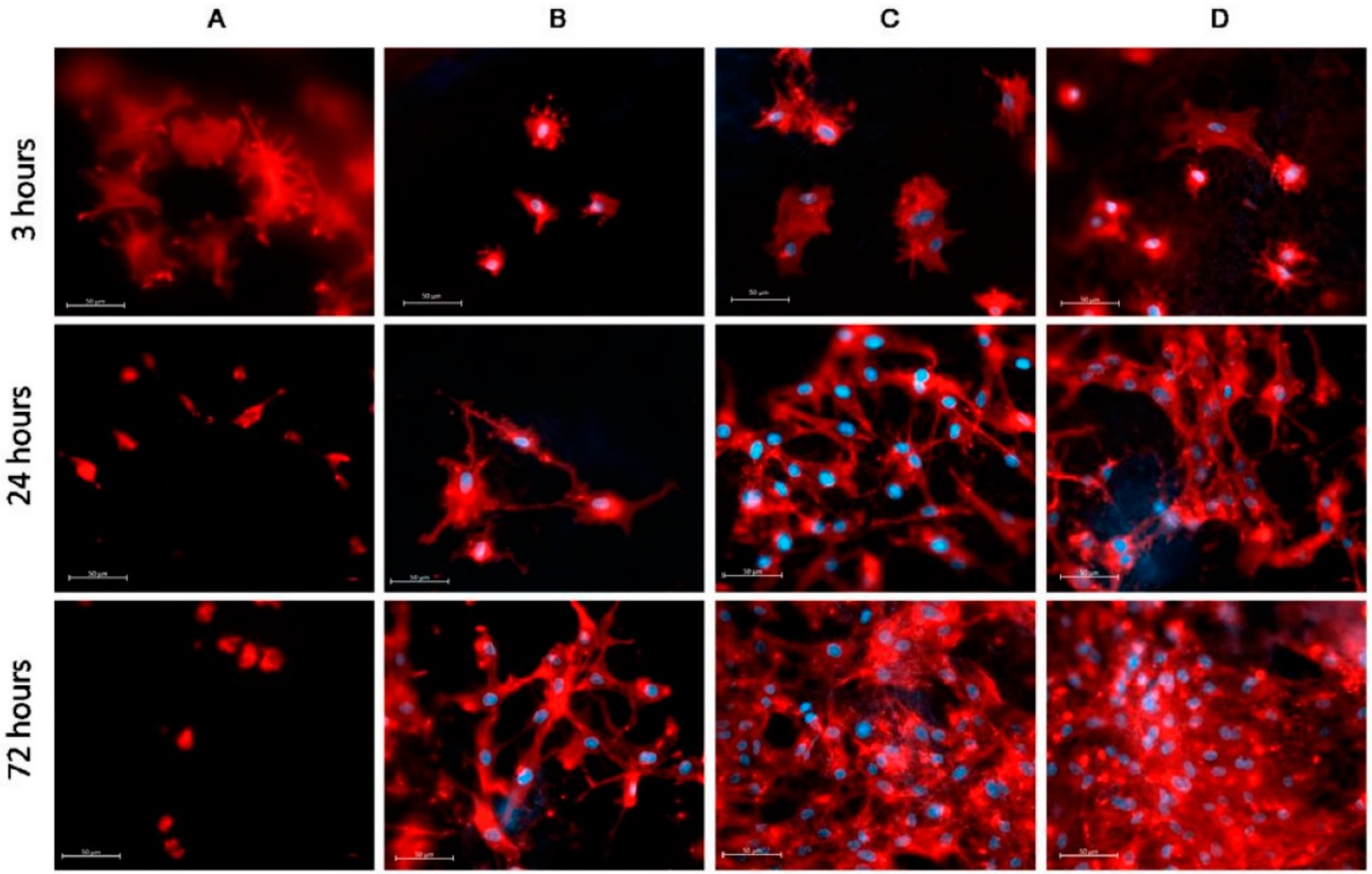


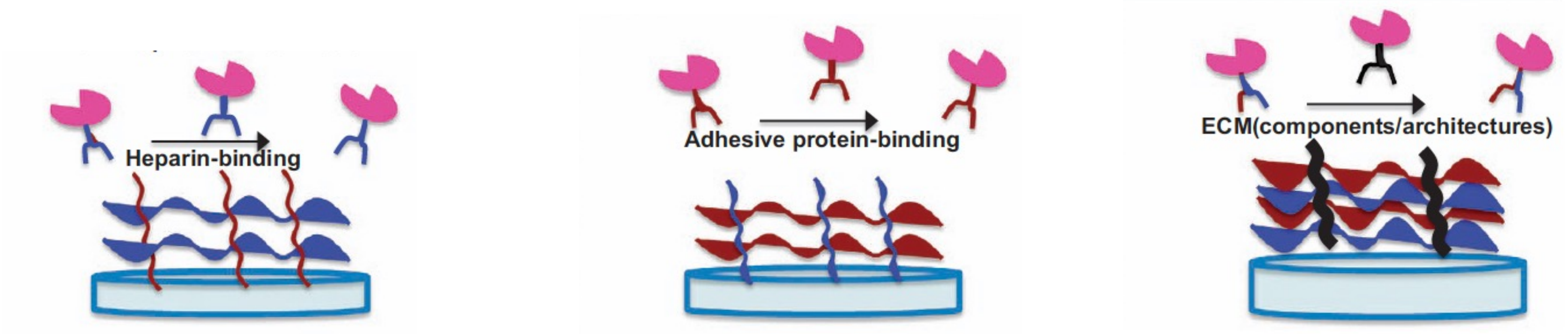
Figure 24. Adhesion of MSCs on the surface of untreated PCL (**A**), COOH-coated PCL (**B**), COOH-coated PCL with physically adsorbed PRP (**C**) and COOH-coated PCL with covalently immobilized PRP (**D**). All images were taken with a magnification of 40× and the scale bar corresponds to 50 µm—reproduced from [294,296]. Copyright Wiley, 2007.

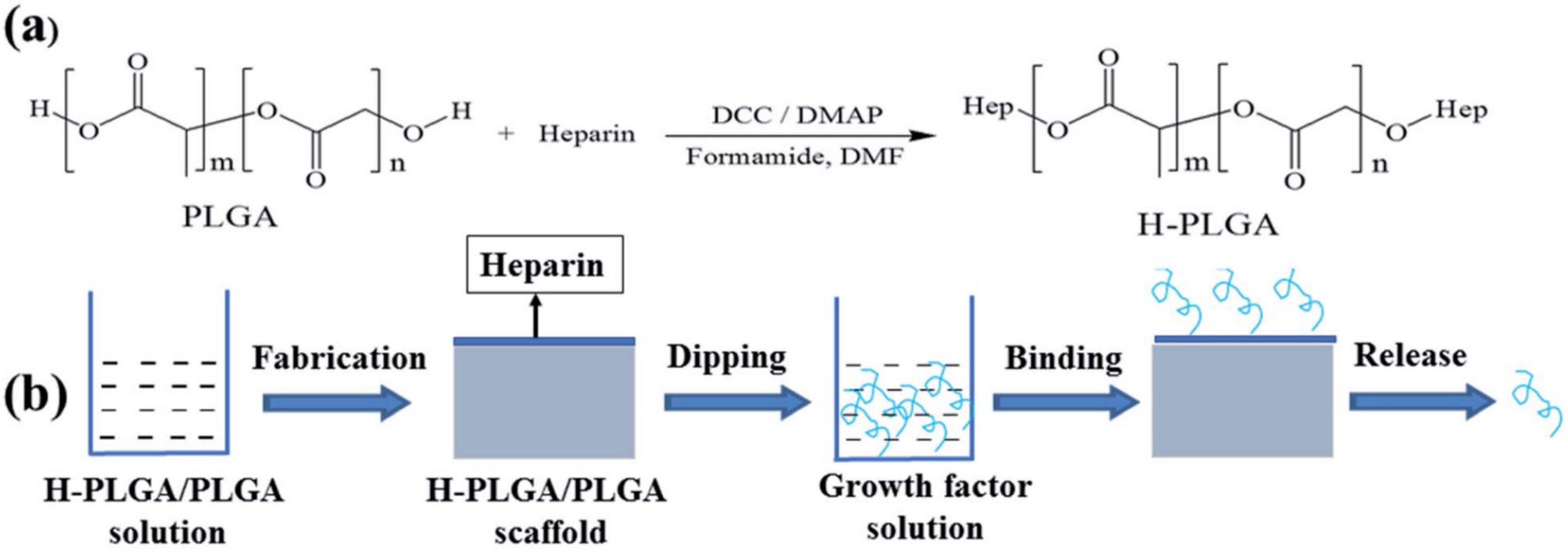
Asadian, Nanomaterials 2020, 10, 119; doi:10.3390/nano10010119

Table 11.1 Biomolecules in tissue engineering

Growth factor	Source	Receptor	Function
Epidermal growth factors (EGFs)	Saliva, plasma, urine and most other body fluids	Tyrosine kinase	Mitogen for ectodermal, mesodermal and endodermal cells, promotes proliferation and differentiation of epidermal and epithelial cells
Fibroblast growth factors (FGFs)	Macrophages, mesenchymal cells, chondrocytes, osteoblasts	Tyrosine kinase	Proliferation of mesenchymal cells, chondrocytes and osteoblasts
Platelet-derived growth factors (PDGFs)	Platelets, macrophages, endothelial cells, fibroblasts, glial cells, astrocytes, myoblasts, smooth muscle cells	Tyrosine kinase	Proliferation of mesenchymal cells, osteoblasts and fibroblasts, macrophage chemotaxis
Insulin-like growth factors (IGFs)	Liver, bone matrix, osteoblasts, chondrocytes, myocytes	Tyrosine kinase	Proliferation and differentiation of osteoprogenitor cells
Transforming growth factor beta (TGF-ß)	Platelets, bone, extracellular matrix	Serine threonine sulfate	Stimulates proliferation of undifferentiated mesenchymal cells
Bone morphogenetic proteins (BMPs)	Bone extracellular matrix, osteoblasts, osteoprogenitor cells	Serine threonine sulfate	Differentiation of -mesenchymal cells into chondrocytes and osteoblasts -osteoprogenitor cells into osteoblasts influences embryonic development

ECM-Inspired Immobilization





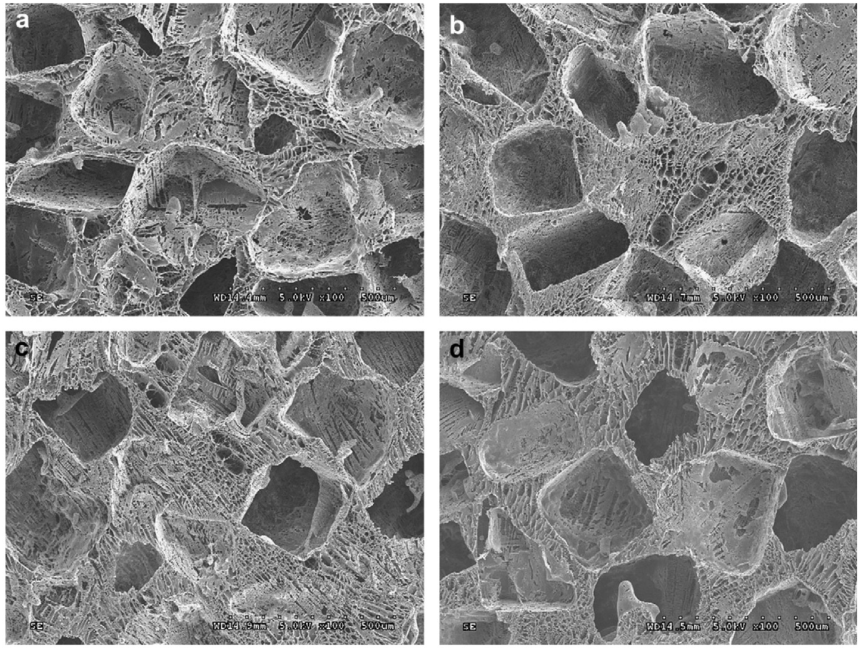
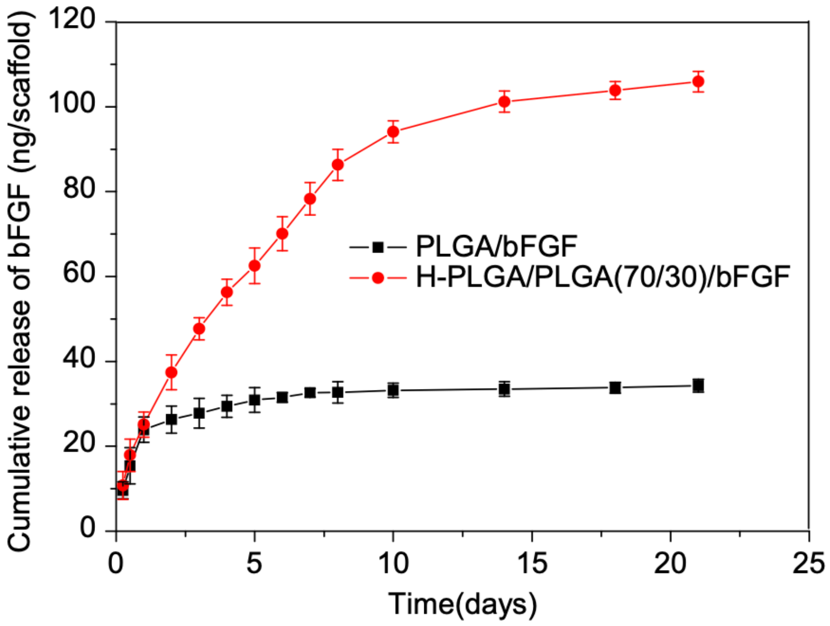
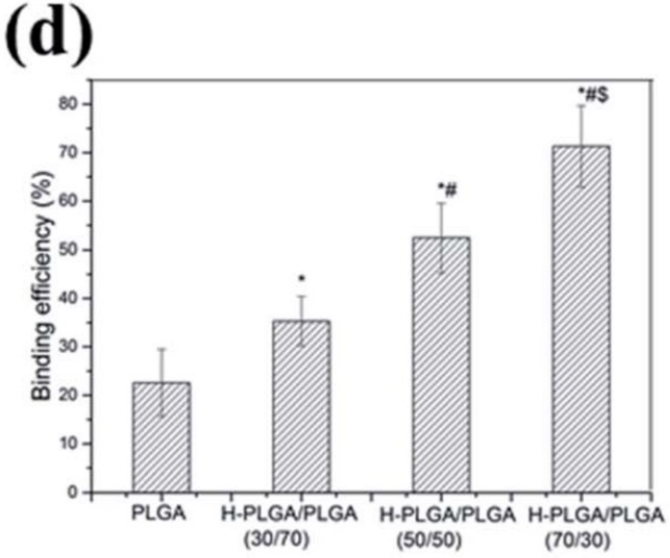
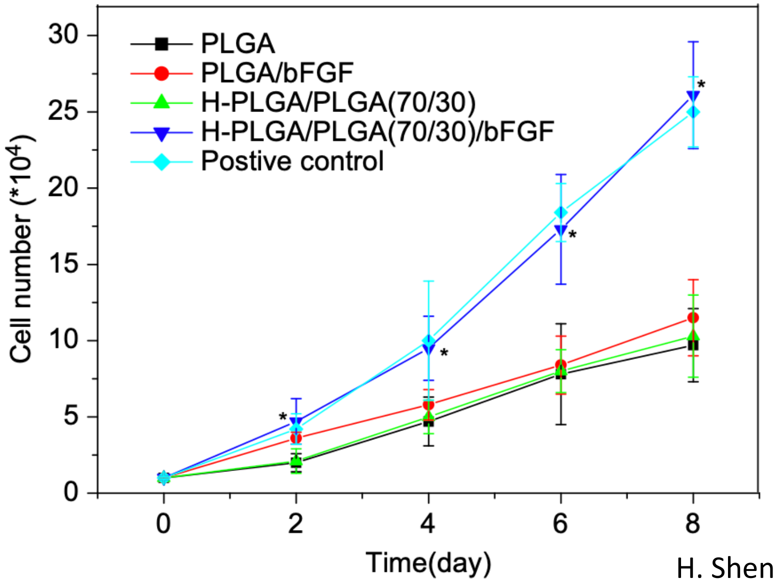


Fig. 2. Morphology structure of PLGA and H-PLGA/PLGA scaffolds. (a) PLGA; (b) H-PLGA/PLGA(30/70); (c) H-PLGA/PLGA(50/50); (d) H-PLGA/PLGA(70/30).

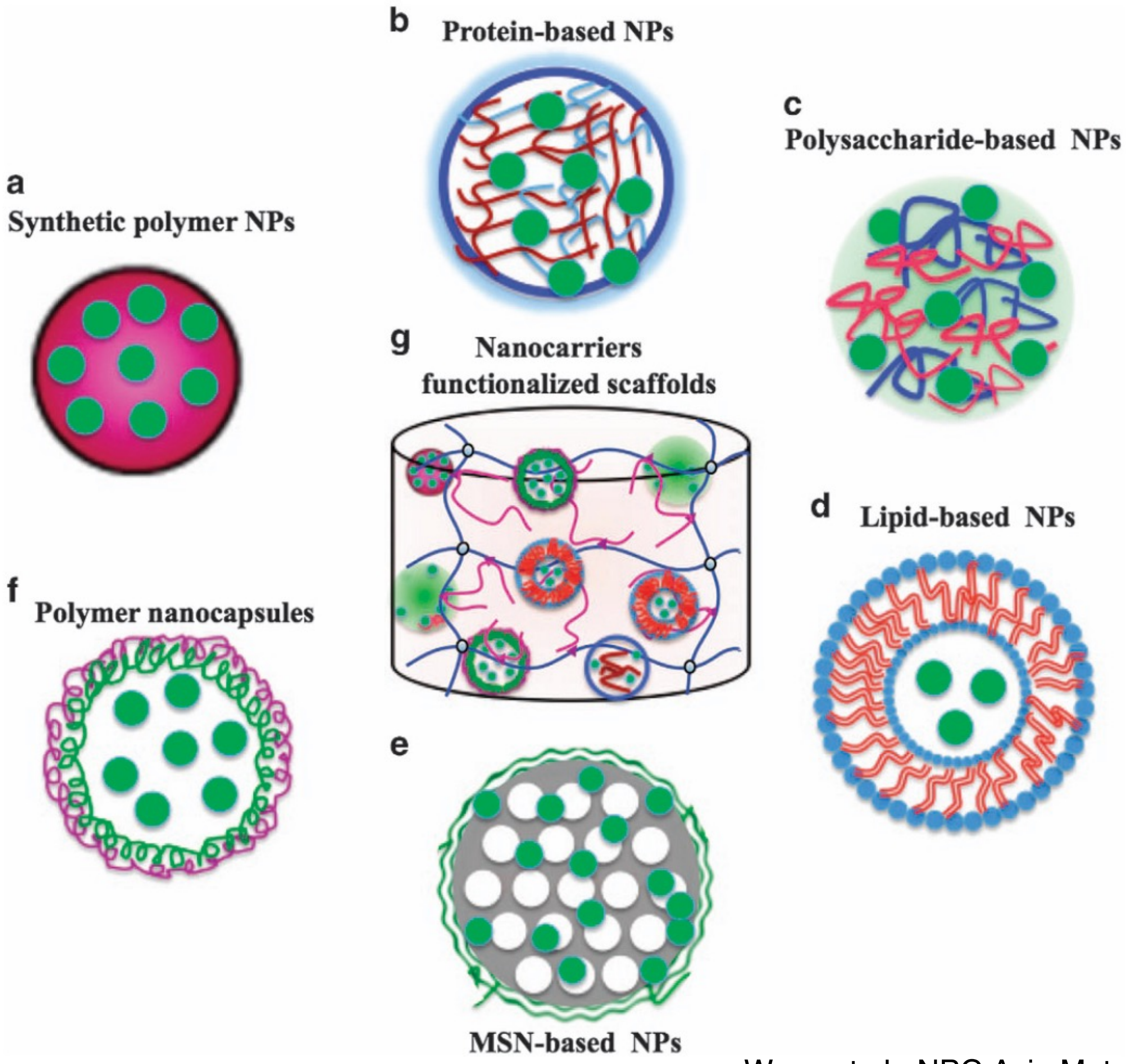




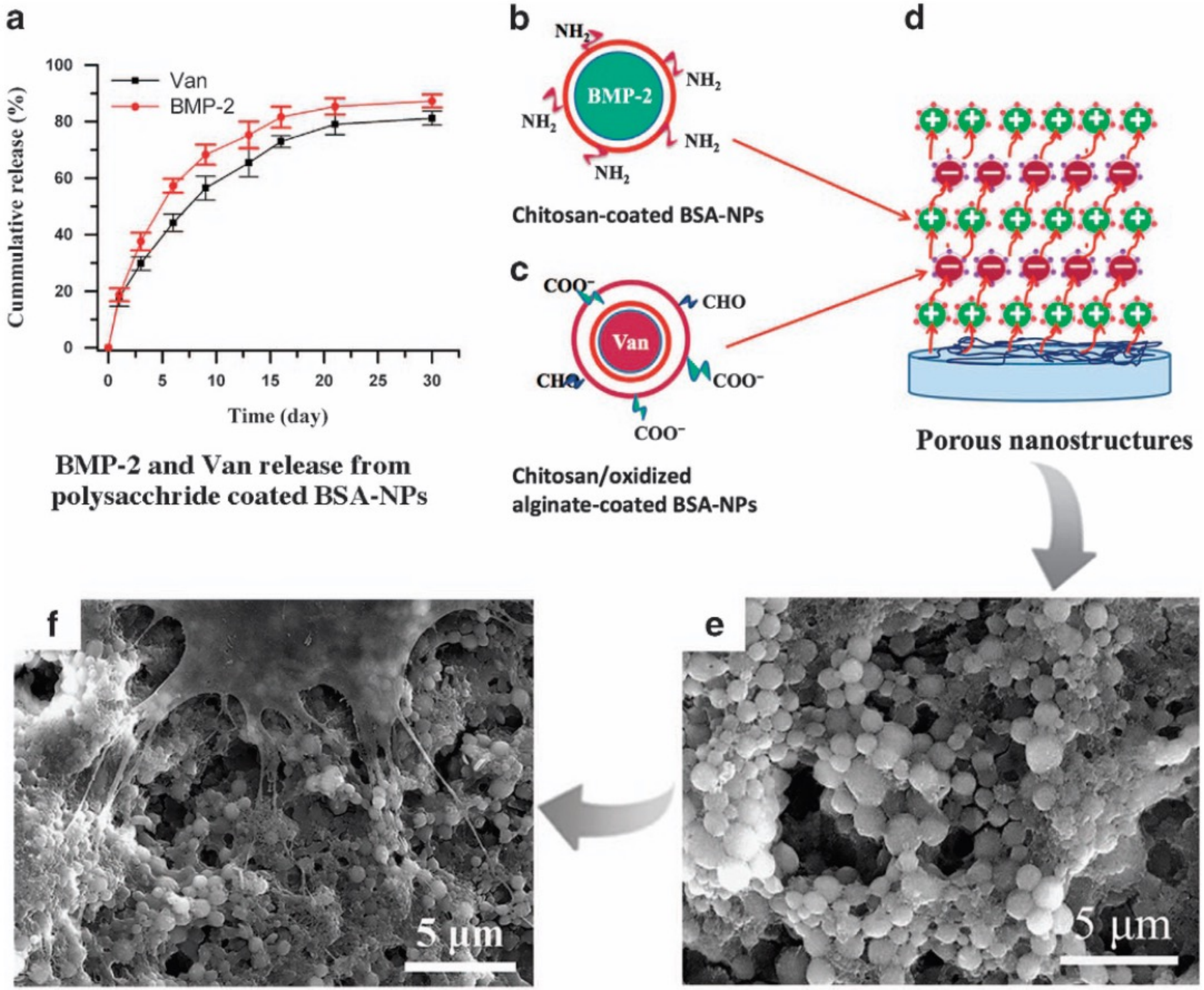


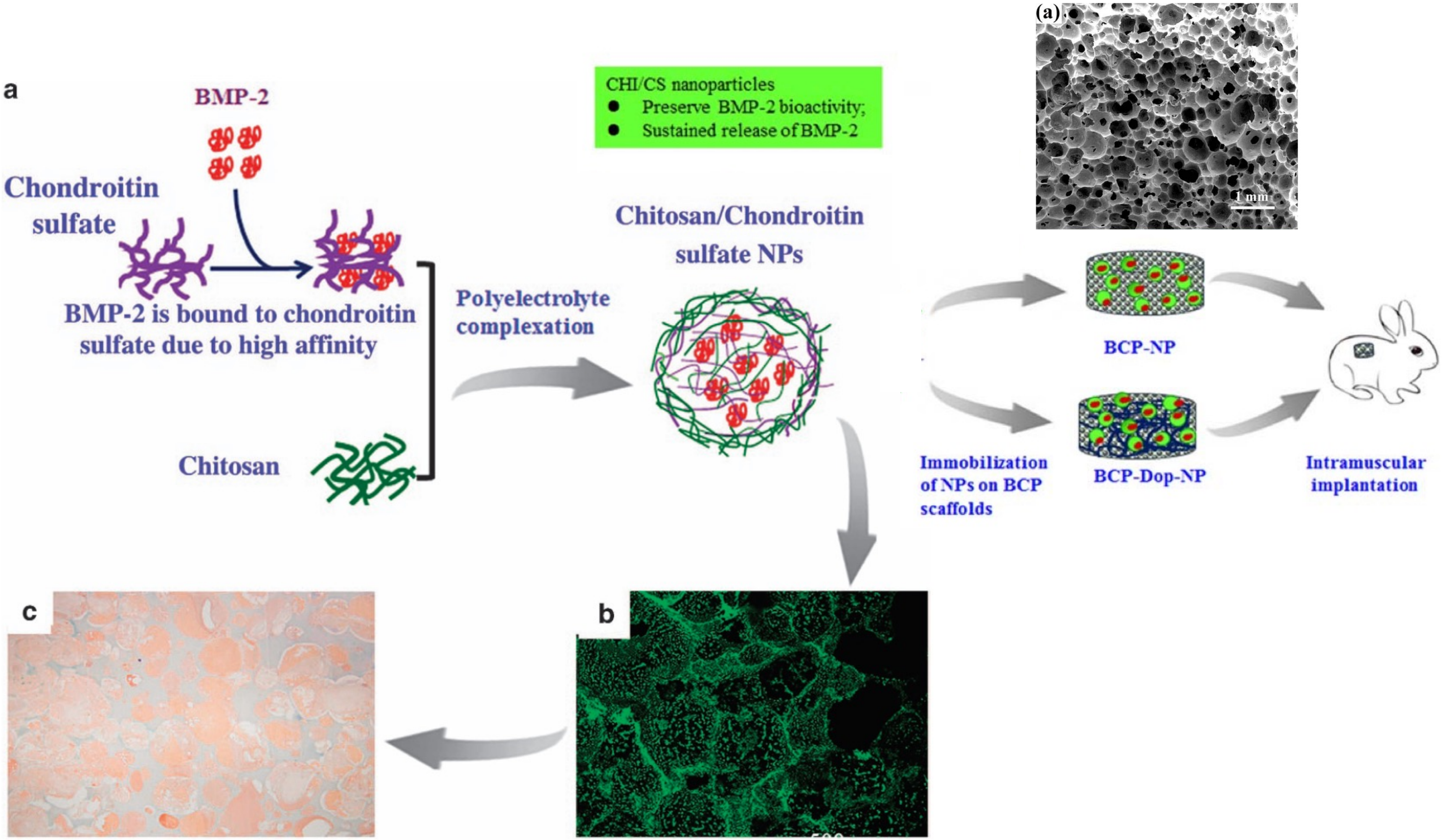
H. Shen et al. / Biomaterials 32 (2011) 3404e3412

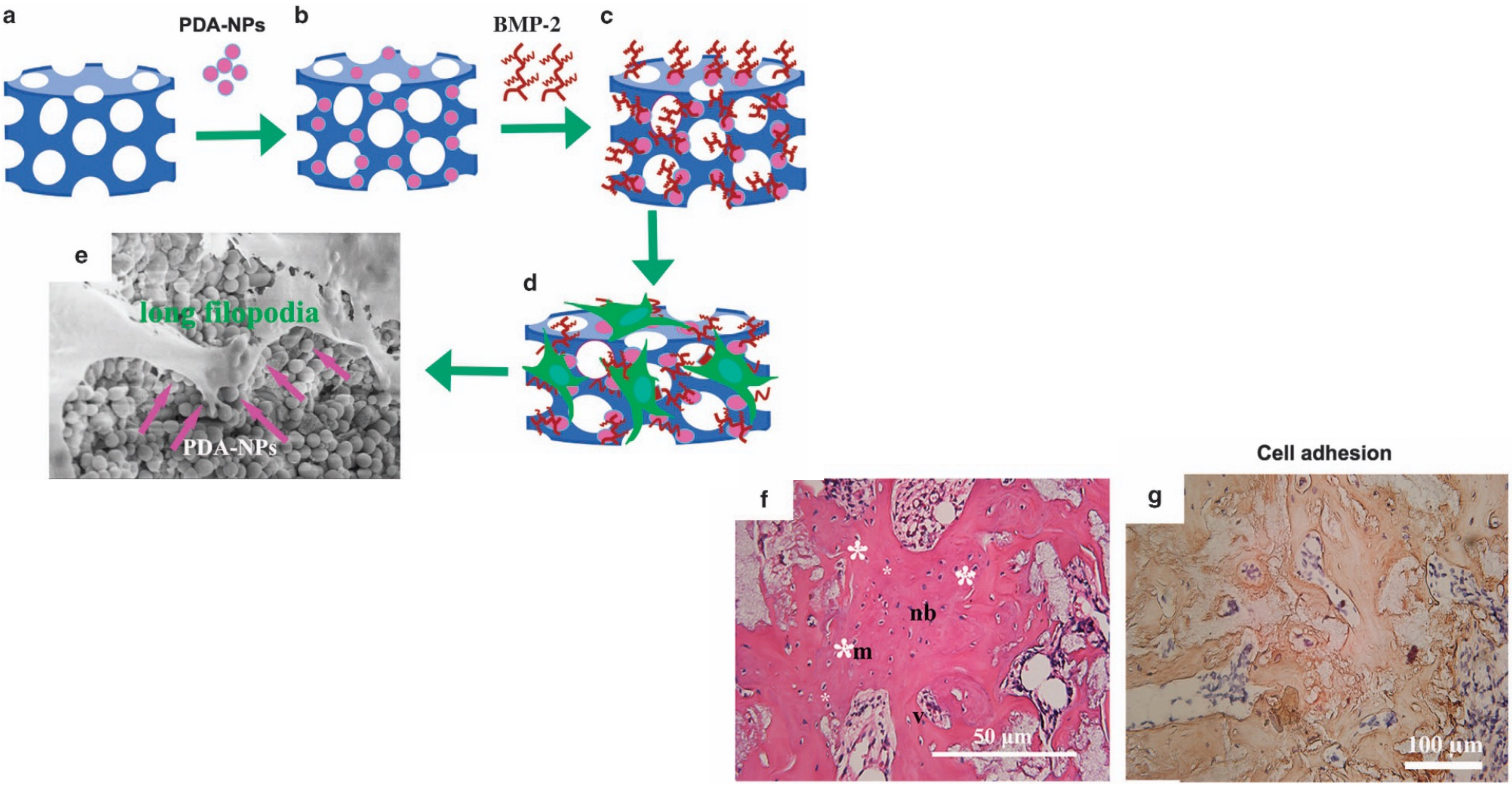
Nanocarriers for GF Encapsulation and release for Biomedical Applications



Wang et al., NPG Asia Materials (2017) 9, e435; doi:10.1038/am.2017.171


a
100
80
60
40
20
0
Cummulative release (%)
Van
BMP-2
0 5 10 15 20 25 30
Time (day)
BMP-2 and Van release from
polysacchride coated BSA-NPs
b
BMP-2
NH2
Chitosan-coated BSA-NPs
c
Van
COO⁻
CHO
Chitosan/oxidized
alginate-coated BSA-NPs
d
Porous nanostructures
e
5 µm
f
5 µm


a
BMP-2
Chondroitin
sulfate
BMP-2 is bound to chondroitin
sulfate due to high affinity
Chitosan
Polyelectrolyte
complexation
CHI/CS nanoparticles
Preserve BMP-2 bioactivity;
Sustained release of BMP-2
Chitosan/Chondroitin
sulfate NPs
(a)
1 mm
BCP-NP
BCP-Dop-NP
Immobilization
of NPs on BCP
scaffolds
Intramuscular
implantation
b
c


a
PDA-NPs
b
BMP-2
c
d
e
long filopodia
PDA-NPs
Cell adhesion
f
nb
m
v
50 μm
g
100 μm

Techniques for the physicochemical analysis of the surface functionalization

Microscopy Techniques

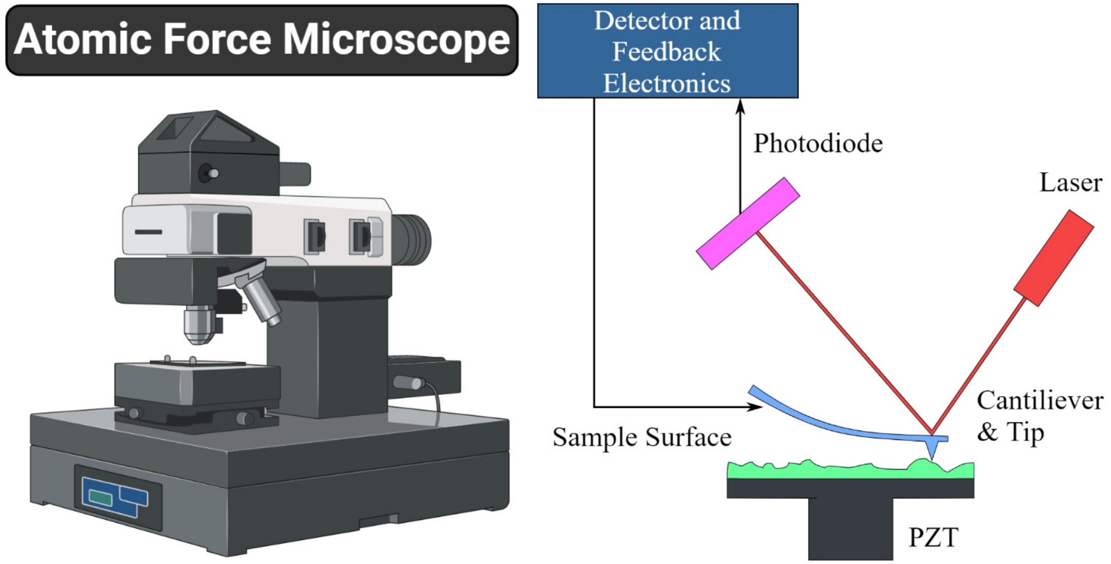
Surface Wettability

Colorimetric Analysis

Spectro-scopy Techniques

Microscopy Techniques

Atomic Force Microscopy (AFM)



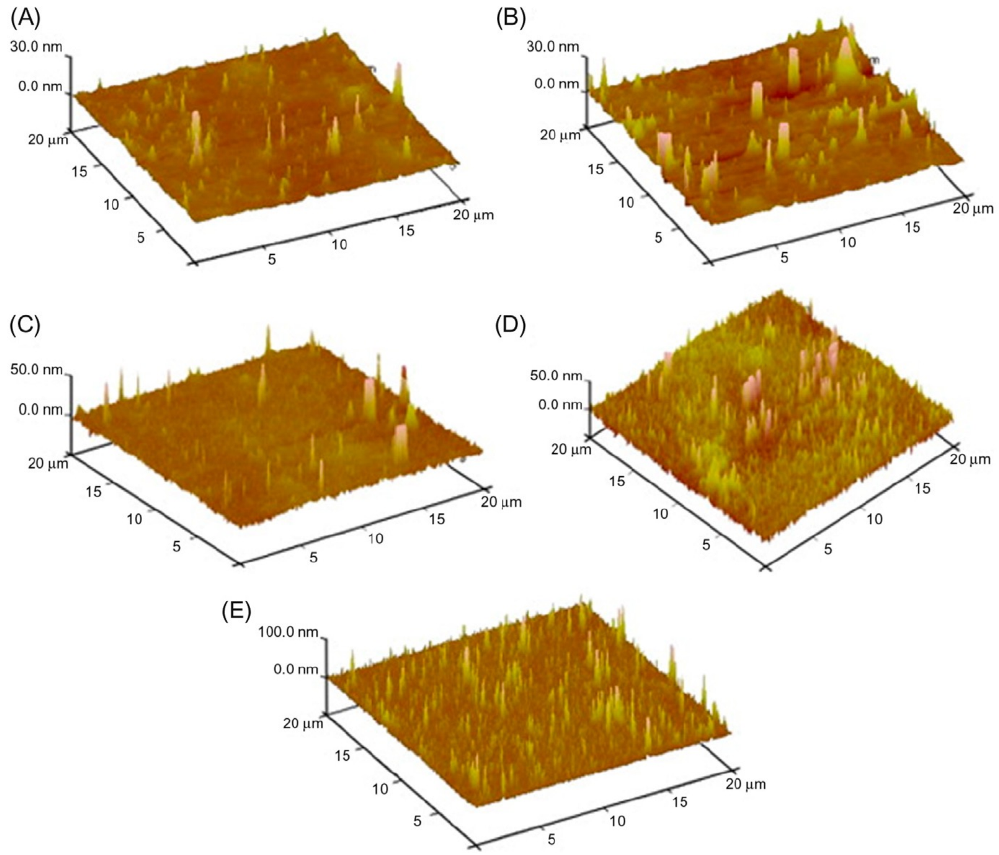
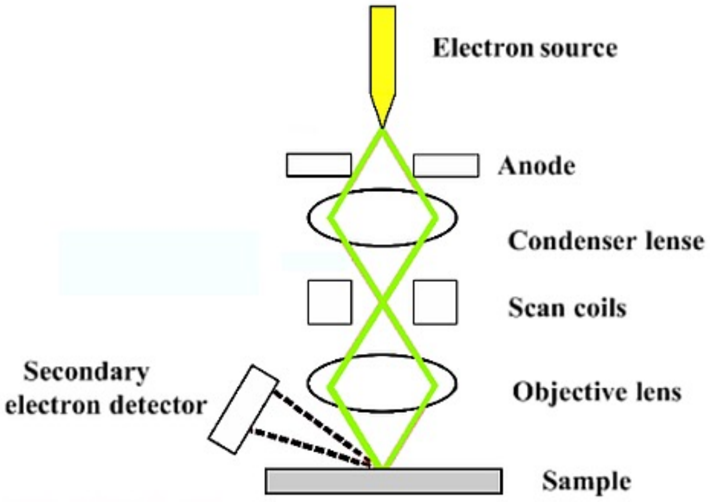


Figure 11.7 AFM topographic of (A) gelatin substrate and gelatin substrates with (B) 1, (C) 6, (D) 9, and (E) 10 layers.

Microscopy Techniques

Scanning Electron Microscopy (SEM)

Scanning Electron Microscope



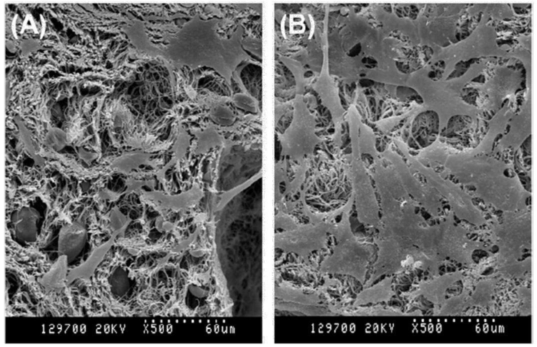
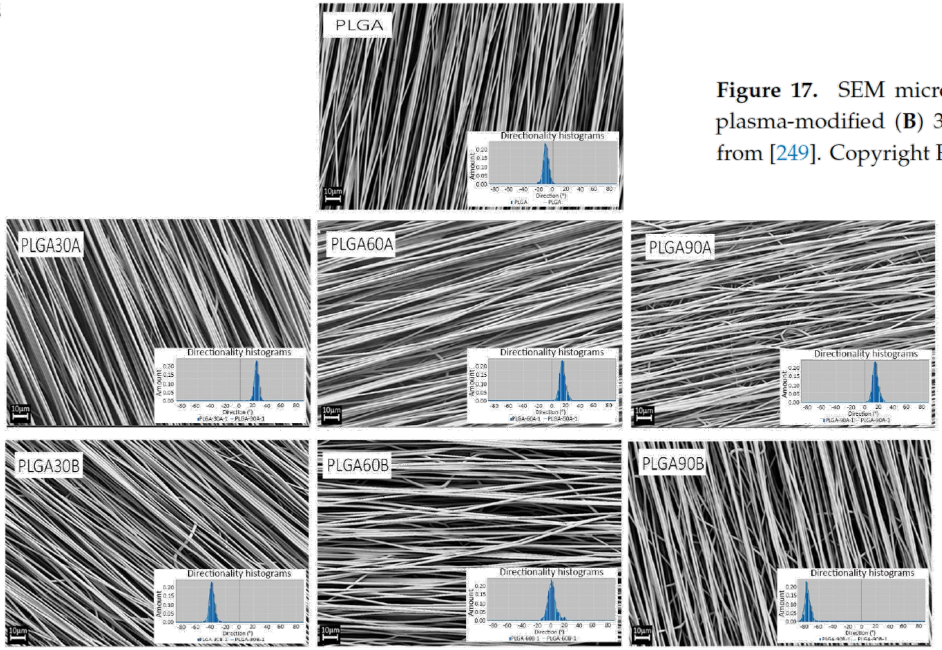
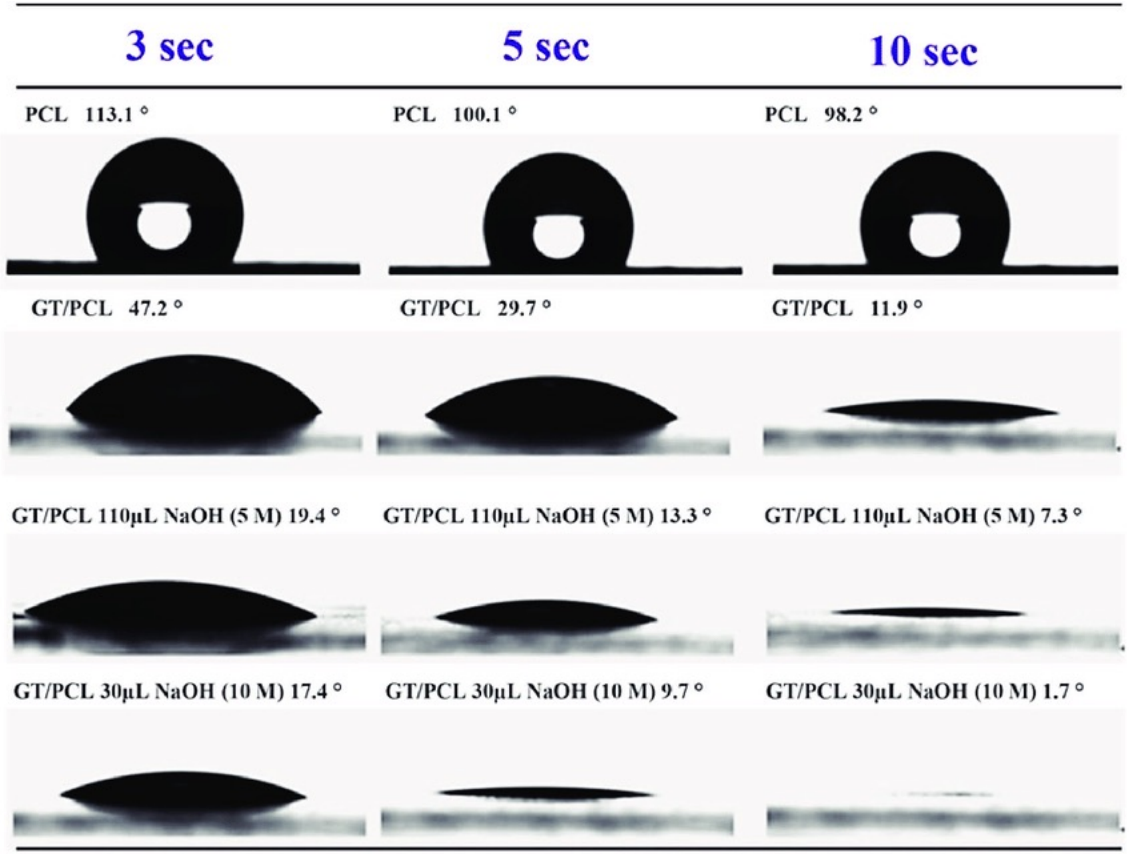


Figure 17. SEM micrographs of nHAC-kn cultured for seven days onto untreated (**A**) and Ar plasma-modified (**B**) 3D porous nanofibrous silk fibroin scaffolds—reproduced with permission from [249]. Copyright Elsevier, 2008.

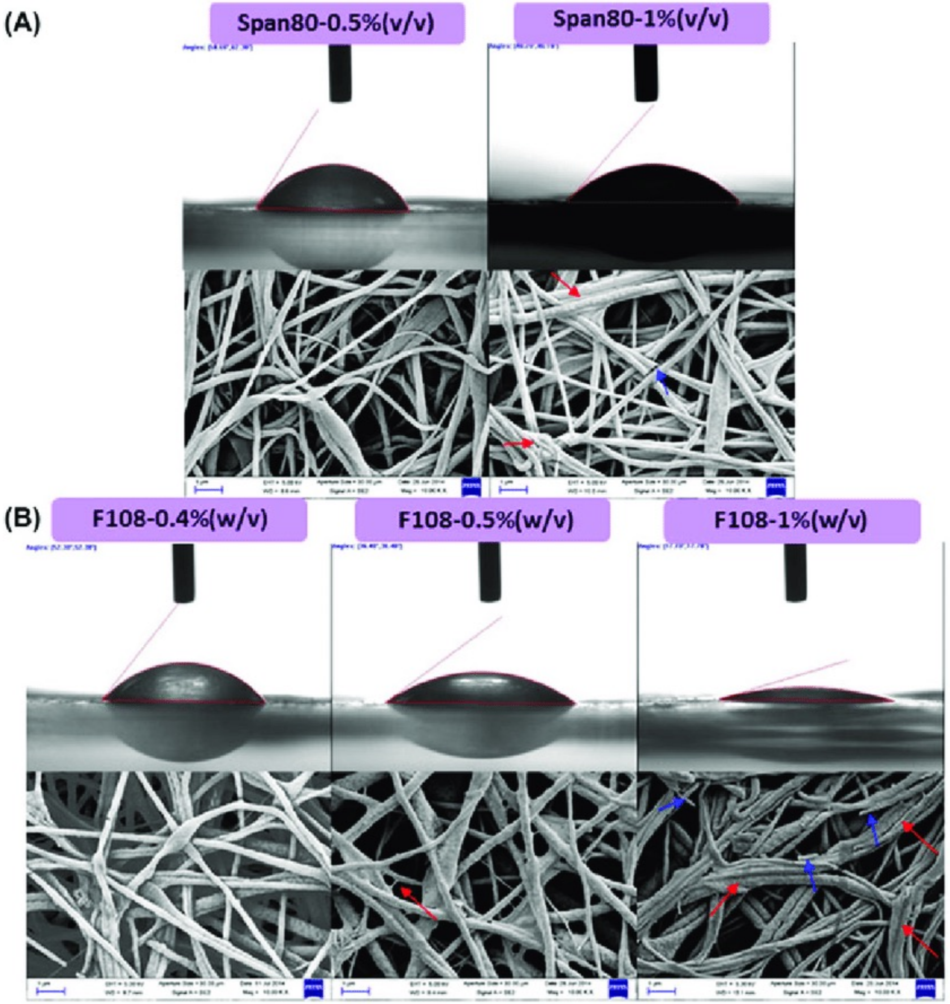


El Khatib et al., Molecules 2020, 25, 3176

Surface Wettability

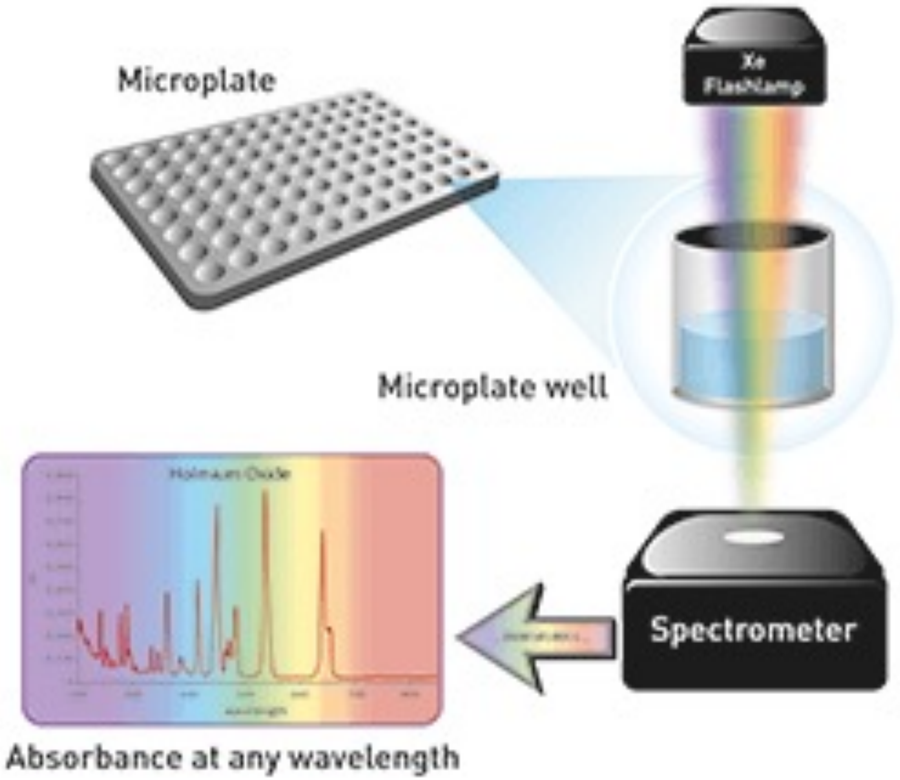


Zhou et al., Macromol. Biosci. 2017, 1700268

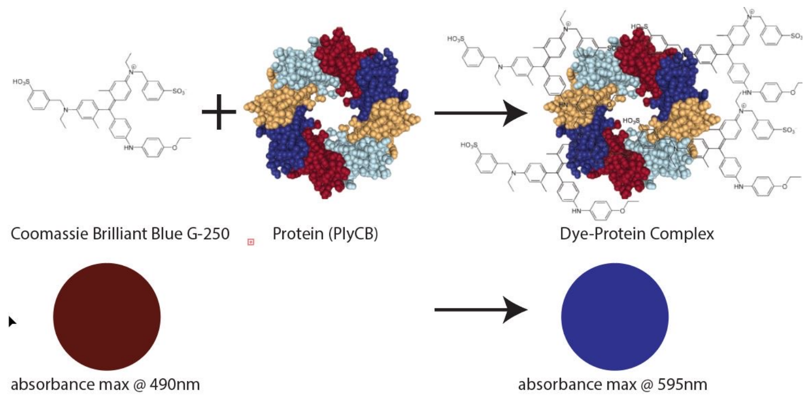


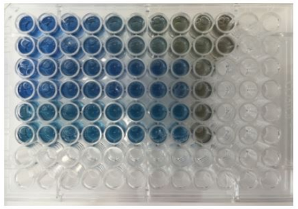
Jue Hu, (2015), Journal of Biomaterial Science, Polymer Edition, 26:1; 57-75

Colorimetric analysis

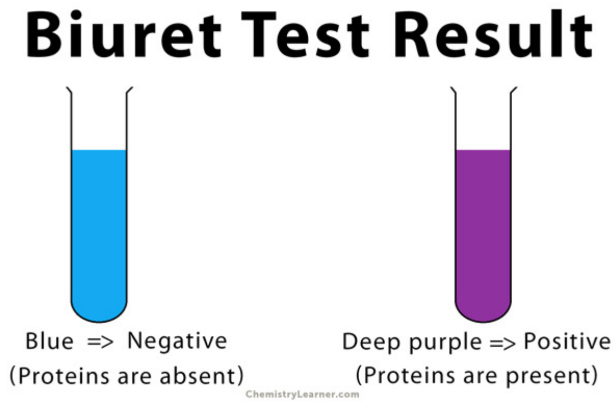


Bradford assay



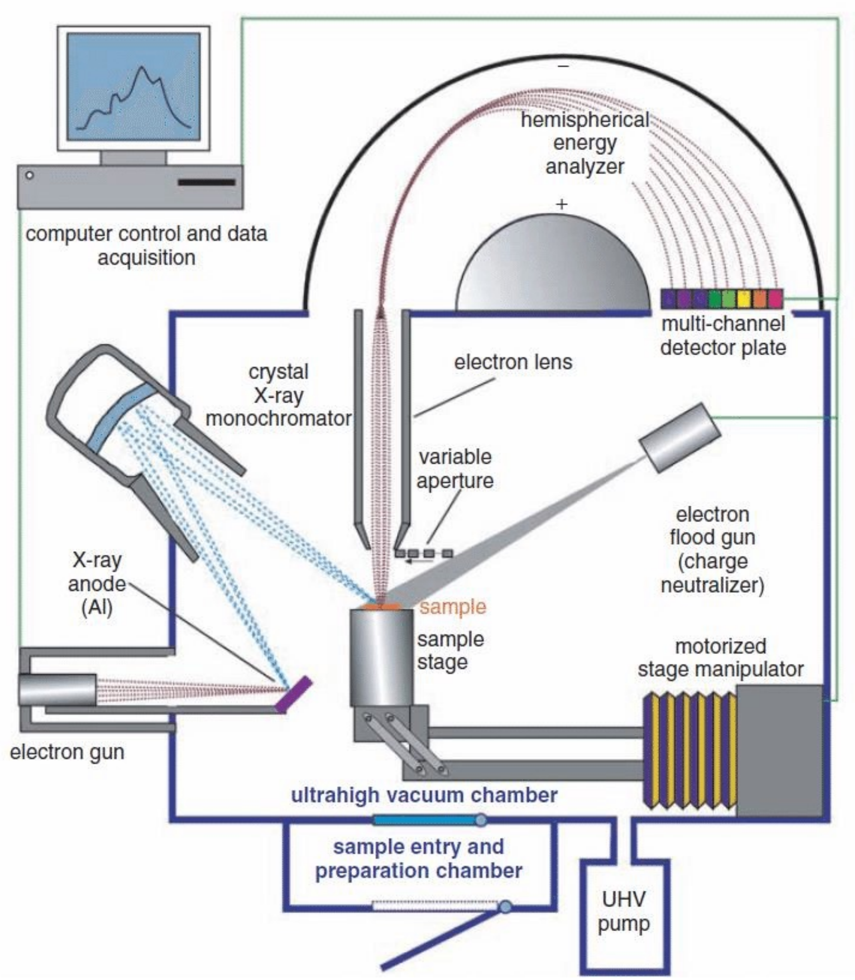


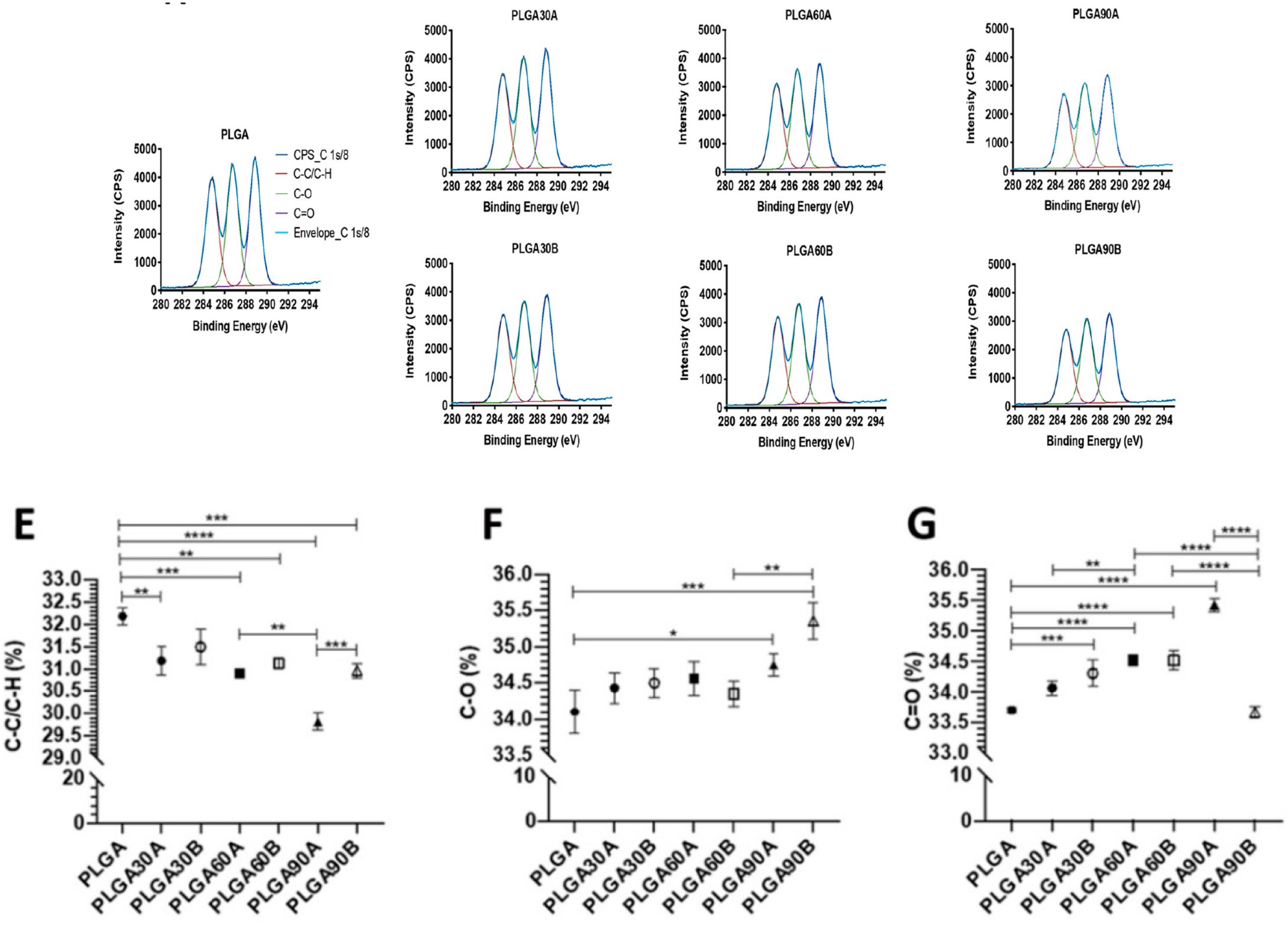
Bradford assay in 96-well plate containing wells with and without protein.



Spectroscopy Techniques

XPS analysis

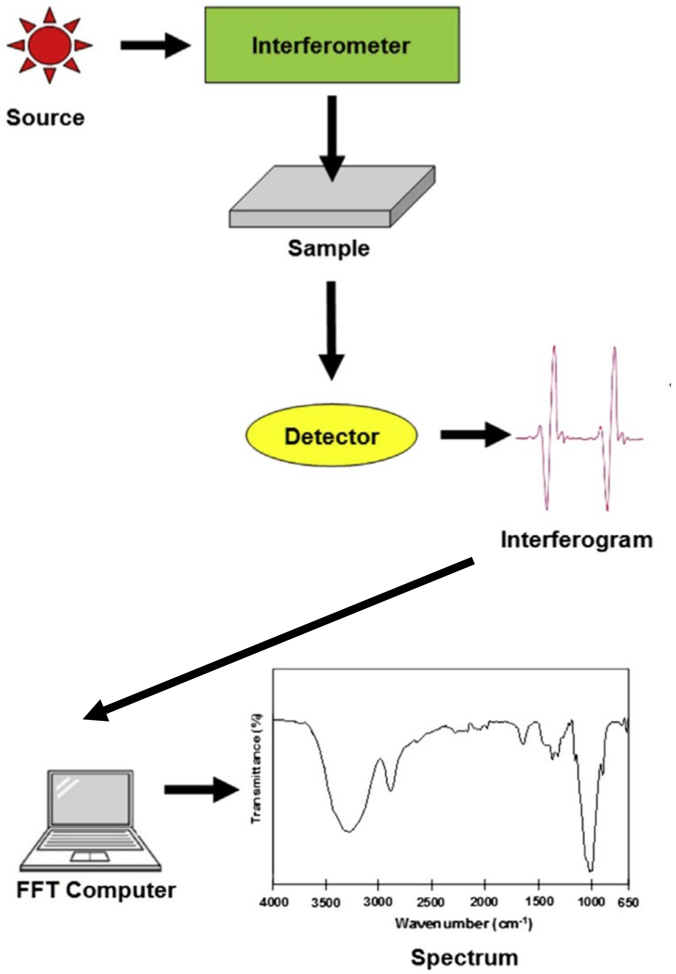




El Khatib et al., Molecules 2020, 25, 3176

Spectroscopy Techniques

FTIR Spectroscopy



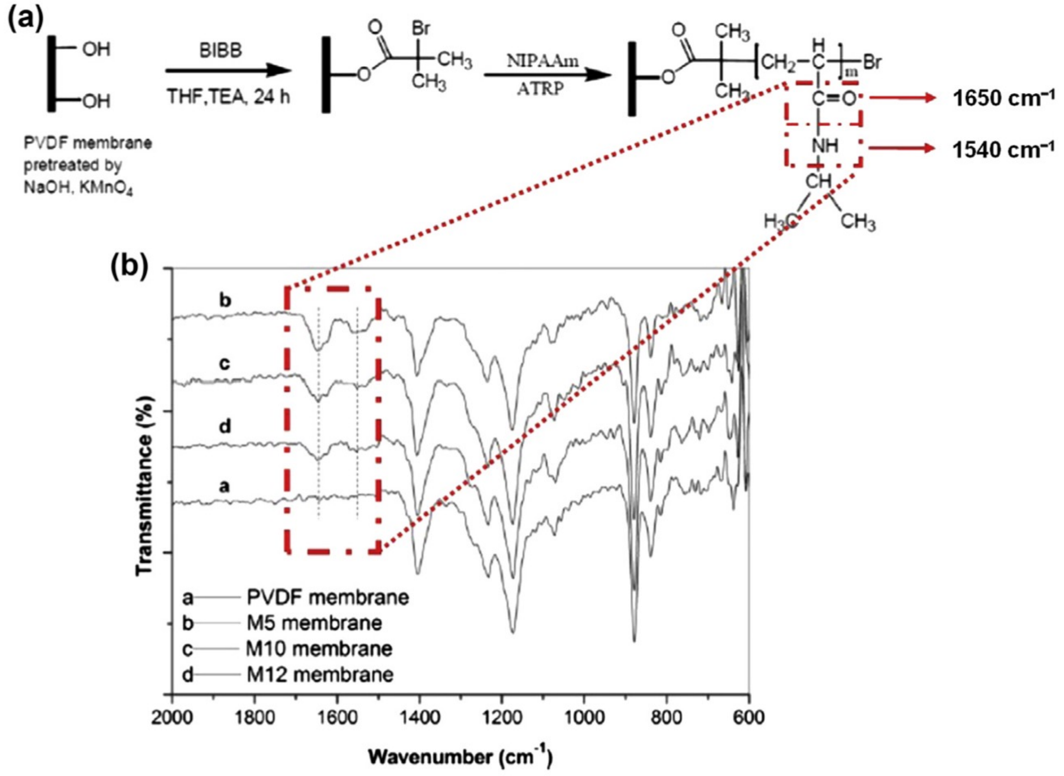


Figure 1.10

(a) Schematic illustration of preparation of modified membrane and (b) attenuated total reflectance-Fourier transform infrared spectra of the pristine and modified poly(vinylidene fluoride) membranes: M5, M10, and M12 membranes with grafting density of 1.17, 0.60, and 0.43 mg/cm^2, respectively. *Reprinted with permission from Zhao G, Chen W-N. Enhanced PVDF membrane performance via surface modification by functional polymer poly(N-isopropylacrylamide) to control protein adsorption and bacterial adhesion. React Funct Polym 2015;97:19–29. Copyright 2015, Elsevier.*