UNIVERSITÁ DEGLI STUDI DI TERAMO

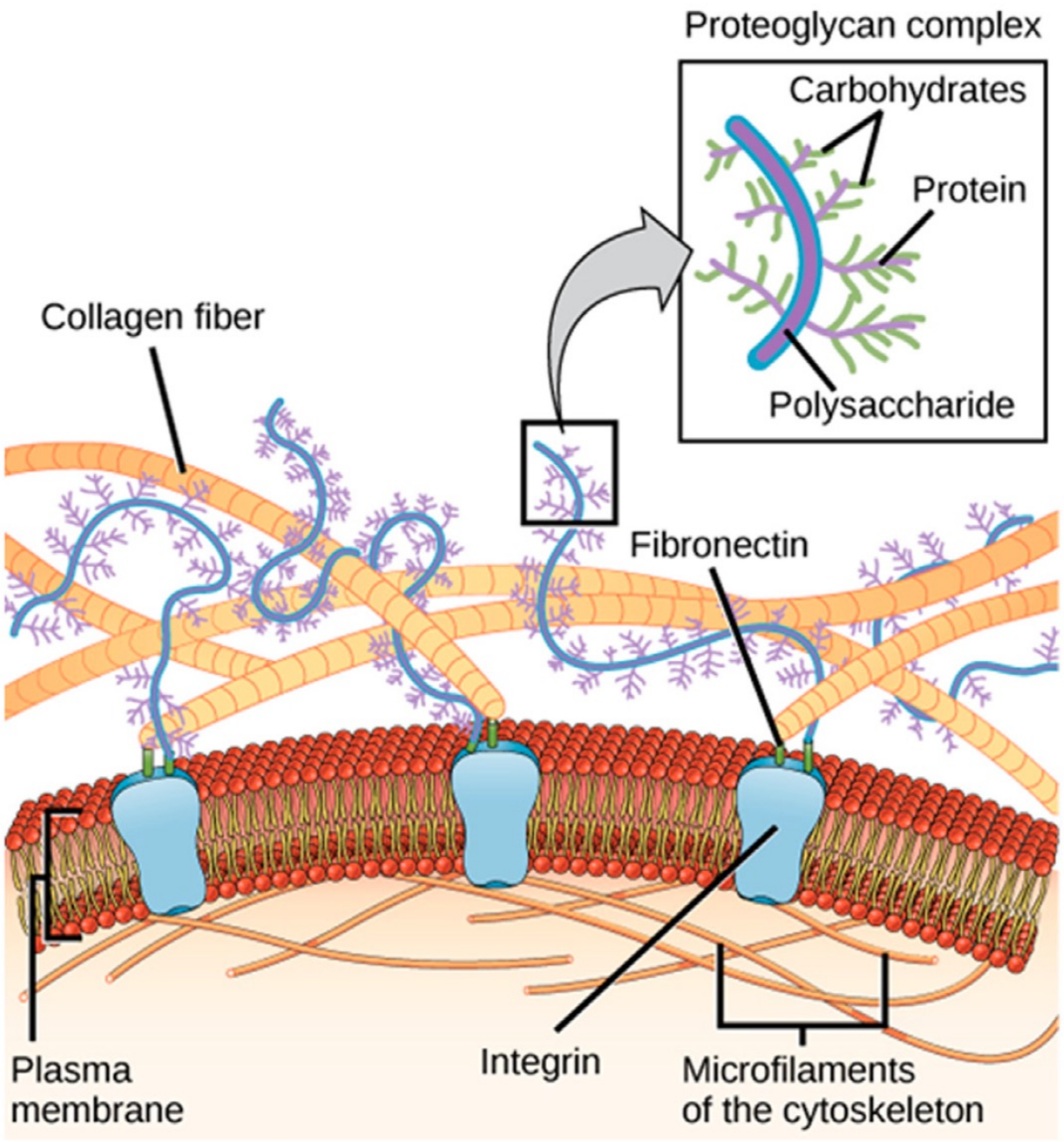
Functionalization Techniques of Scaffolds for TE Applications

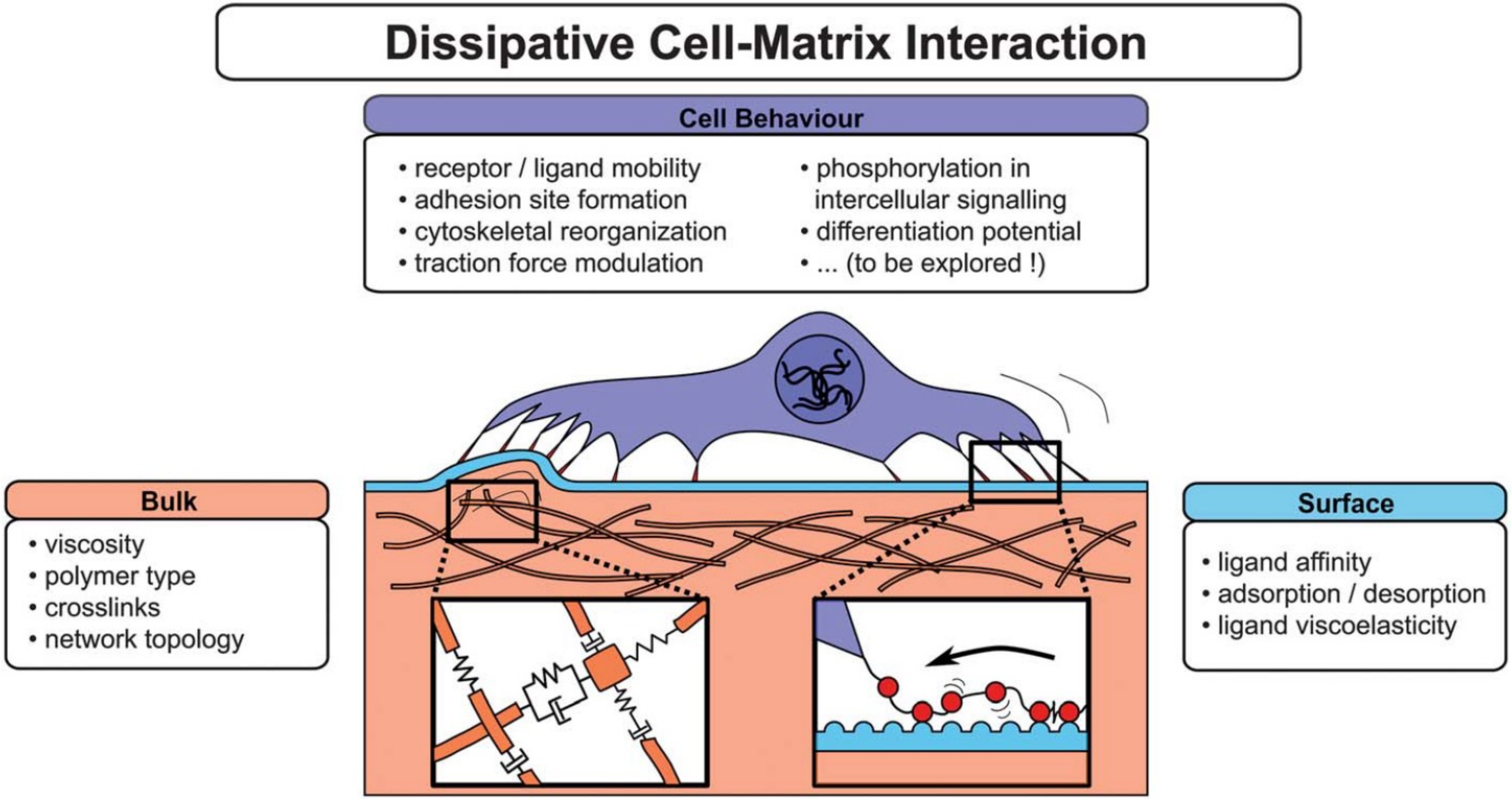
- Prepare scaffolds with biomimetic properties in respect to those of the **ECM of the tissue to be engineered**, including: biomimetic mechanical properties, chemical composition, and architecture.

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

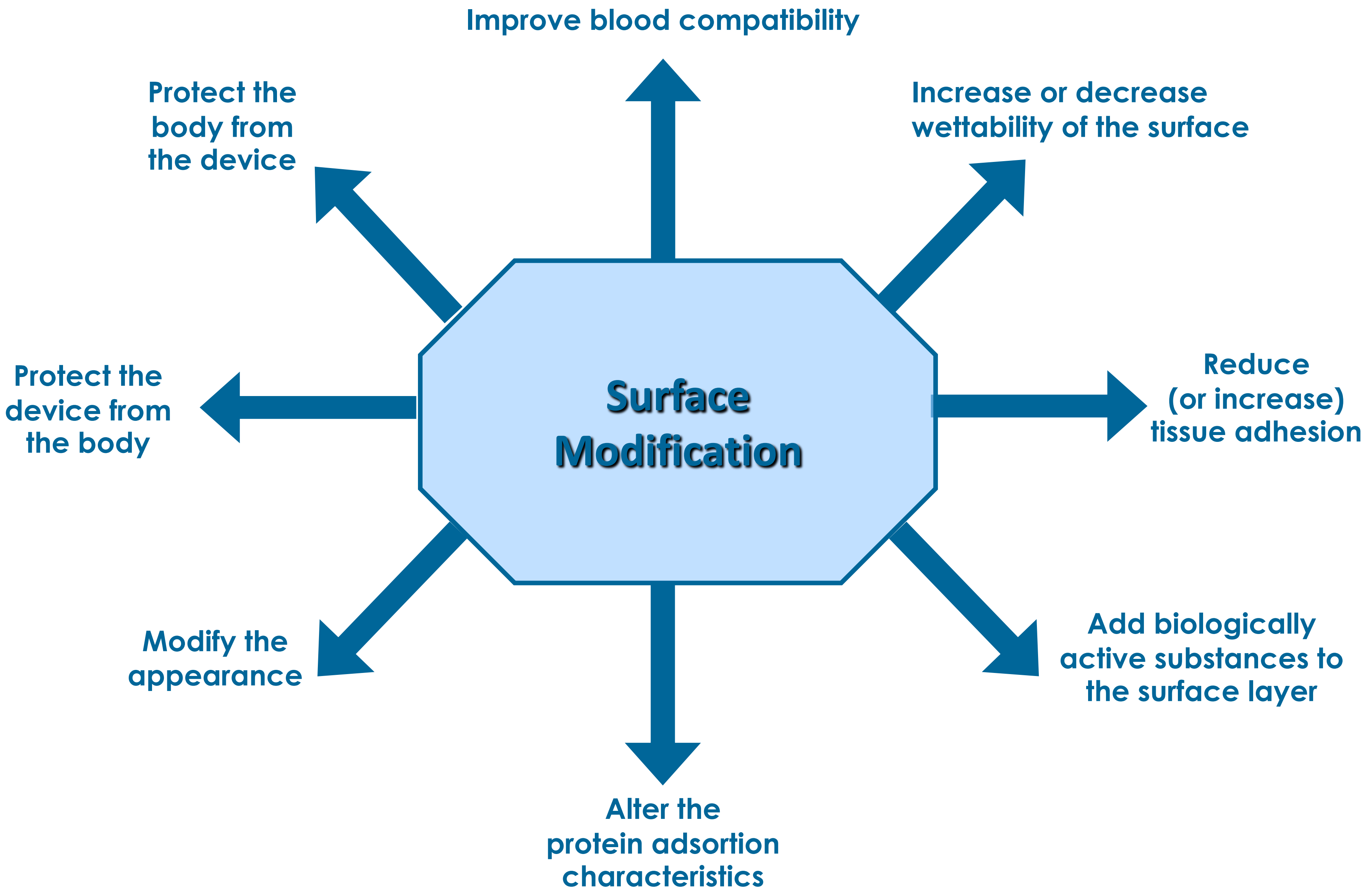
- **Glycosaminoglycans** and **proteoglycans** mainly regulate the level of hydration of natural ECM, its permeability and the traffic and activity of soluble molecules secreted by cells.

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





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

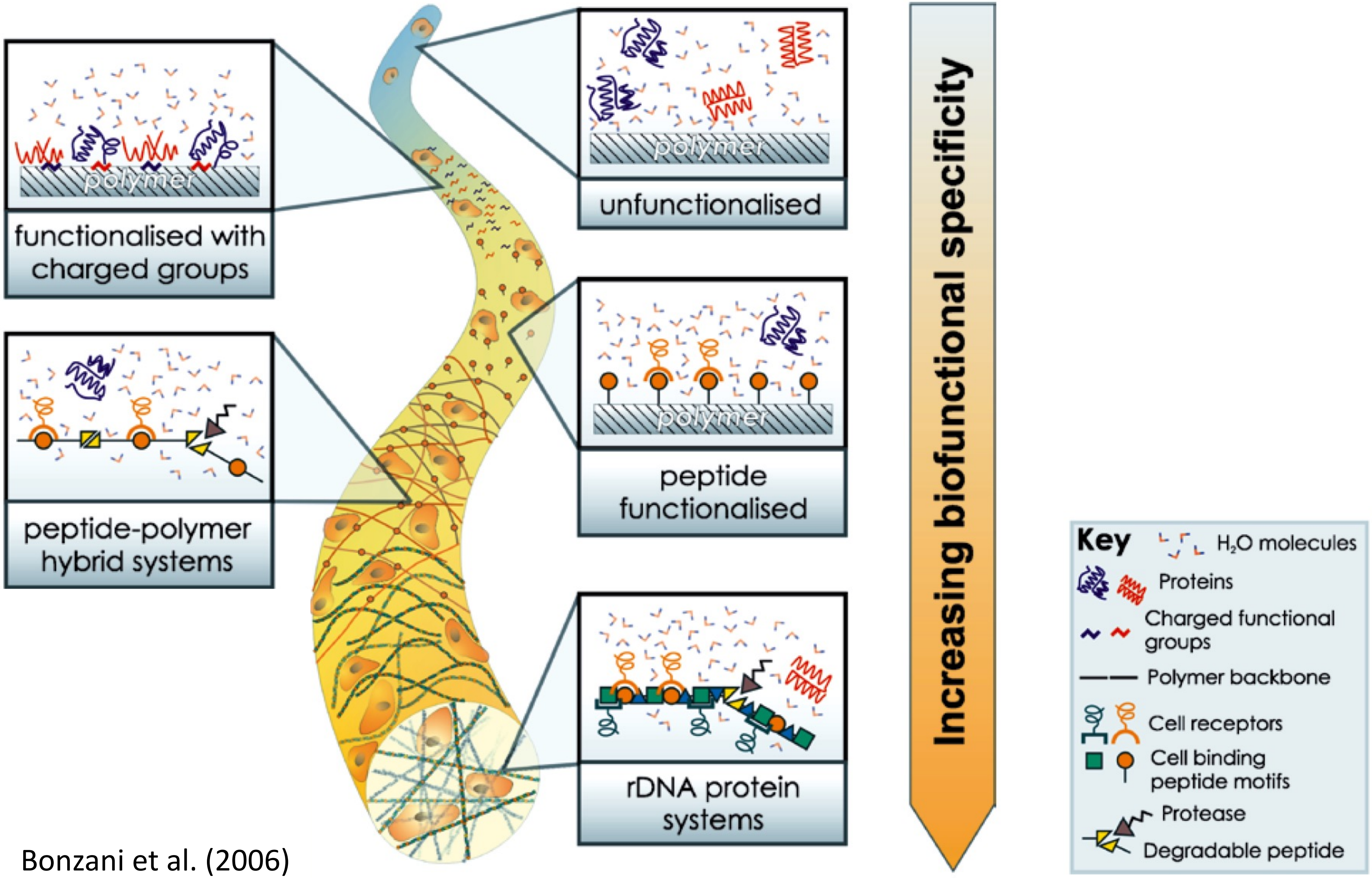

Improve blood compatibility
Protect the body from the device
Increase or decrease wettability of the surface
Protect the device from the body
Surface Modification
Reduce (or increase) tissue adhesion
Modify the appearance
Add biologically active substances to the surface layer
Alter the protein adsortion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

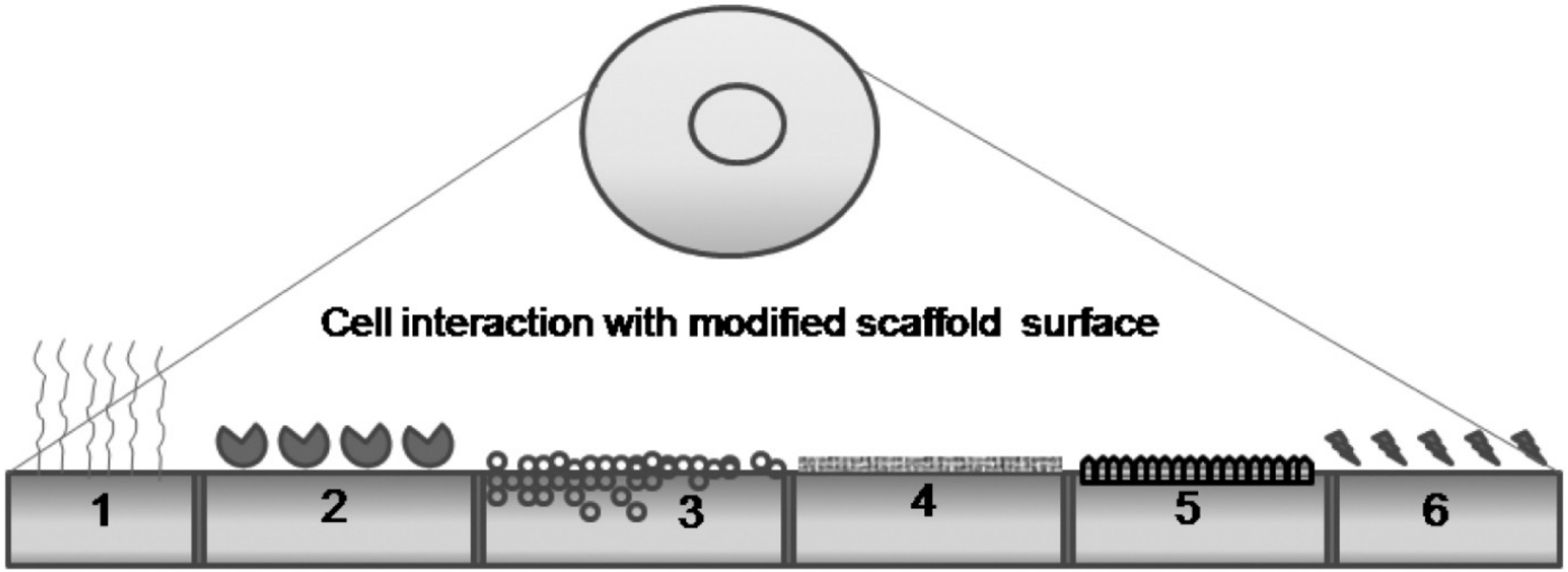
Tallawi et al., 2015, Interface 12: 20150254.
http://dx.doi.org/10.1098/rsif.2015.0254



Bonzani et al. (2006)

Table 11.1 Biomolecules in tissue engineering

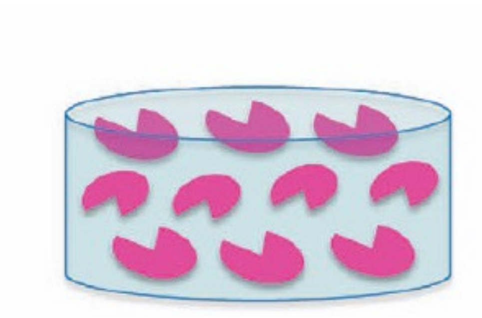
Growth factor	Source	Receptor	Function
Epidermal growth factors (EGFs)	Saliva, plasma, urine and most other body fluids	Tyrosine kinase	Mitogen for ectodermal, mesodermal and endodermal cells, promotes proliferation and differentiation of epidermal and epithelial cells
Fibroblast growth factors (FGFs)	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



1. Introduction of new functional groups by covalent/noncovalent modification (Kāllrot et al., 2008)
2. Attachment of cell adhesion motiffs eg) RGD peptides (Soner Çakmak et al., 2013)
3. Introduction of bioactive molecules (selvakumar et al., 2013)
4. Plasma treatment for inducing hydrophilicity (Cheng et al., 2013)
5. Nanoscale texturing (www.exogenesis.us/2012)
6. Laser modification of scaffolds (Hakeam et al., 2013)

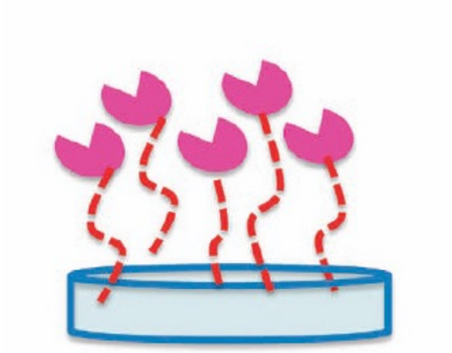
Physical encapsulation/immobilization

1



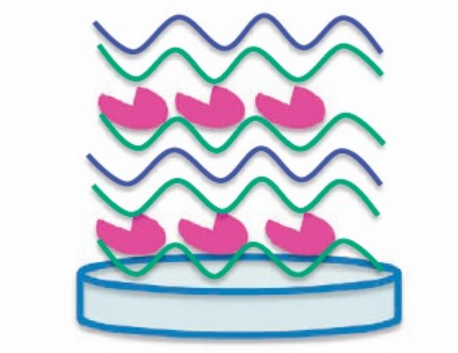
Physical encapsulation of GFs

2

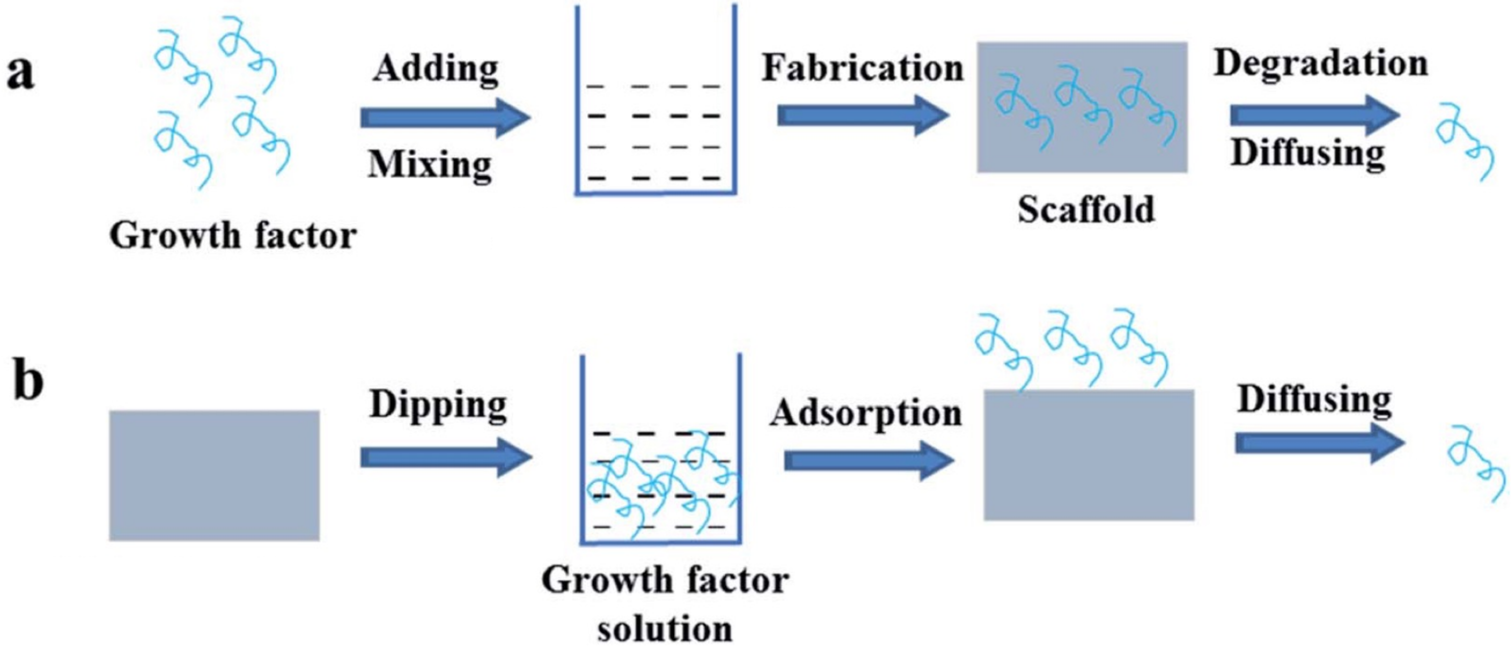


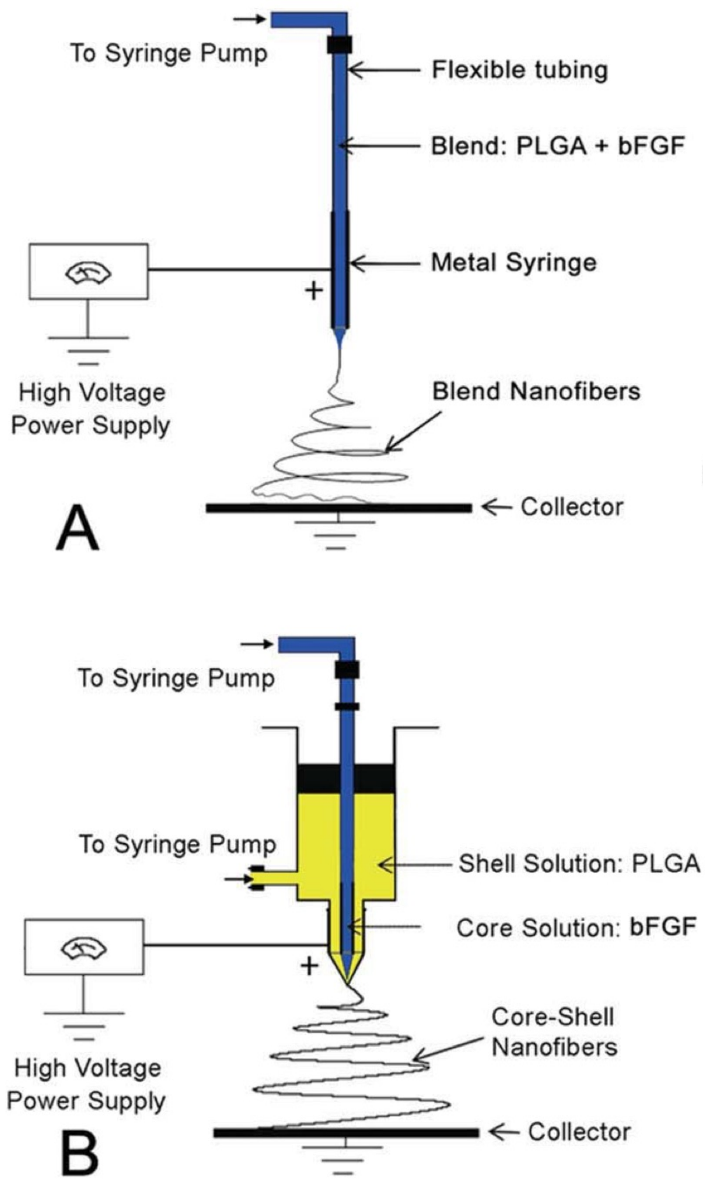
Absorption of GFs on the surface of the scaffolds

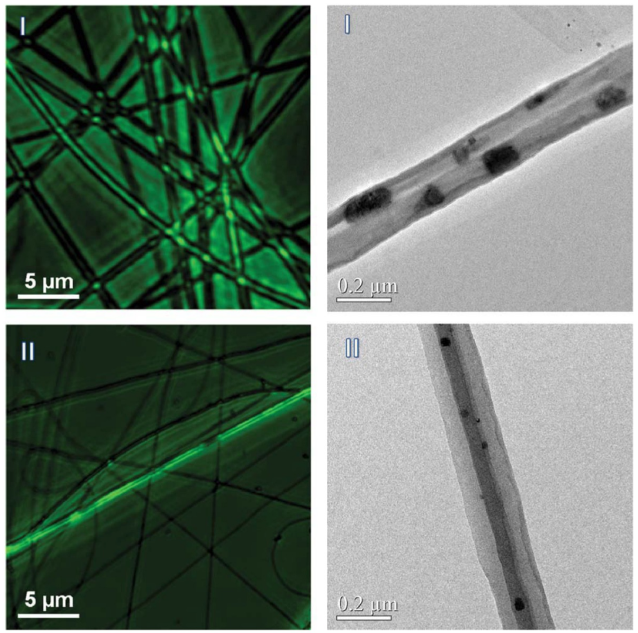
3

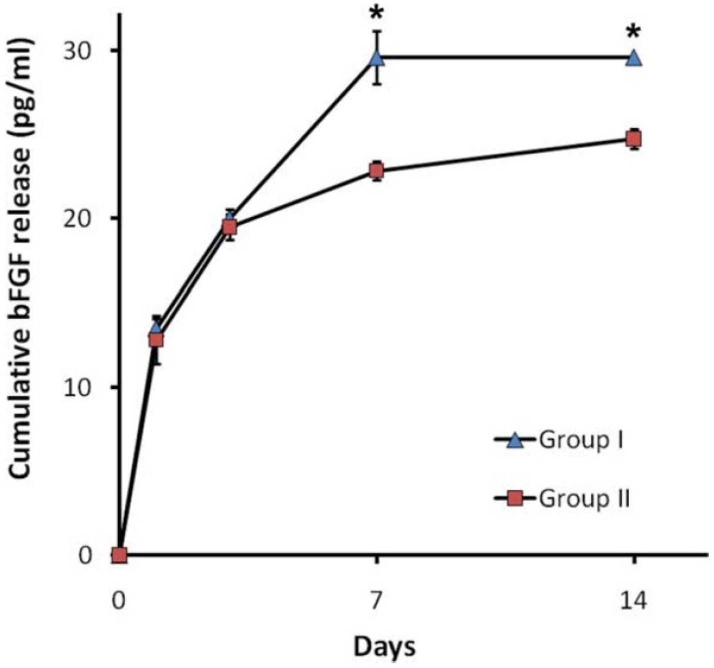


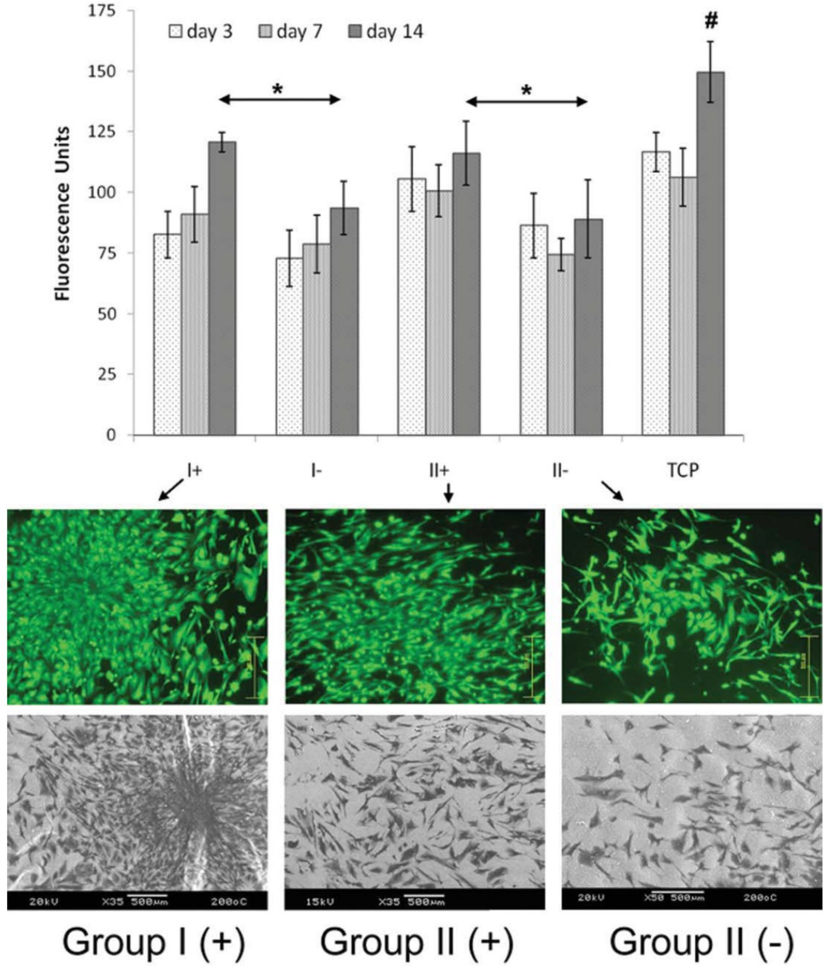
Layer-by-Layer Self Assembly





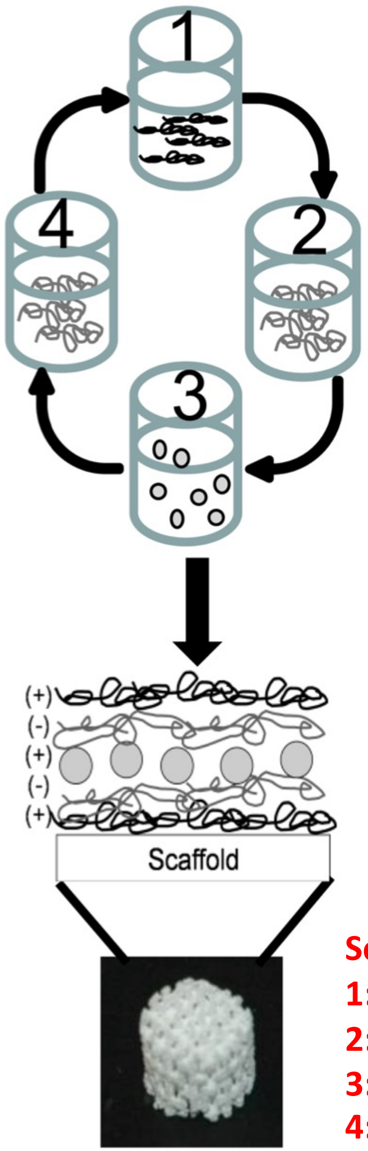


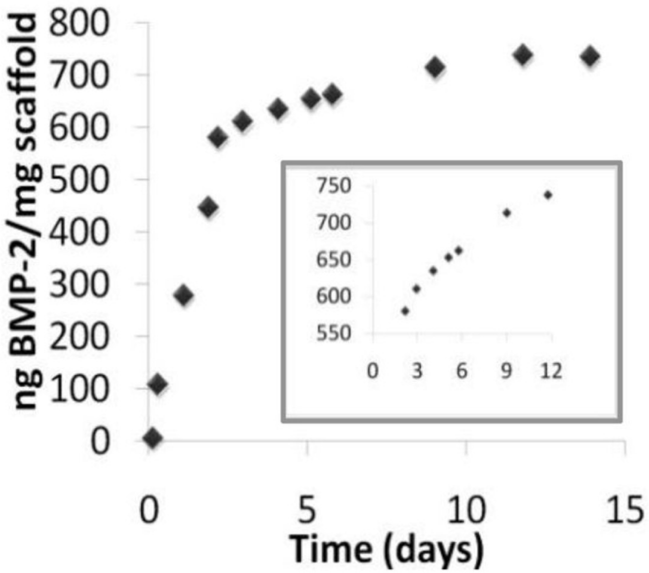


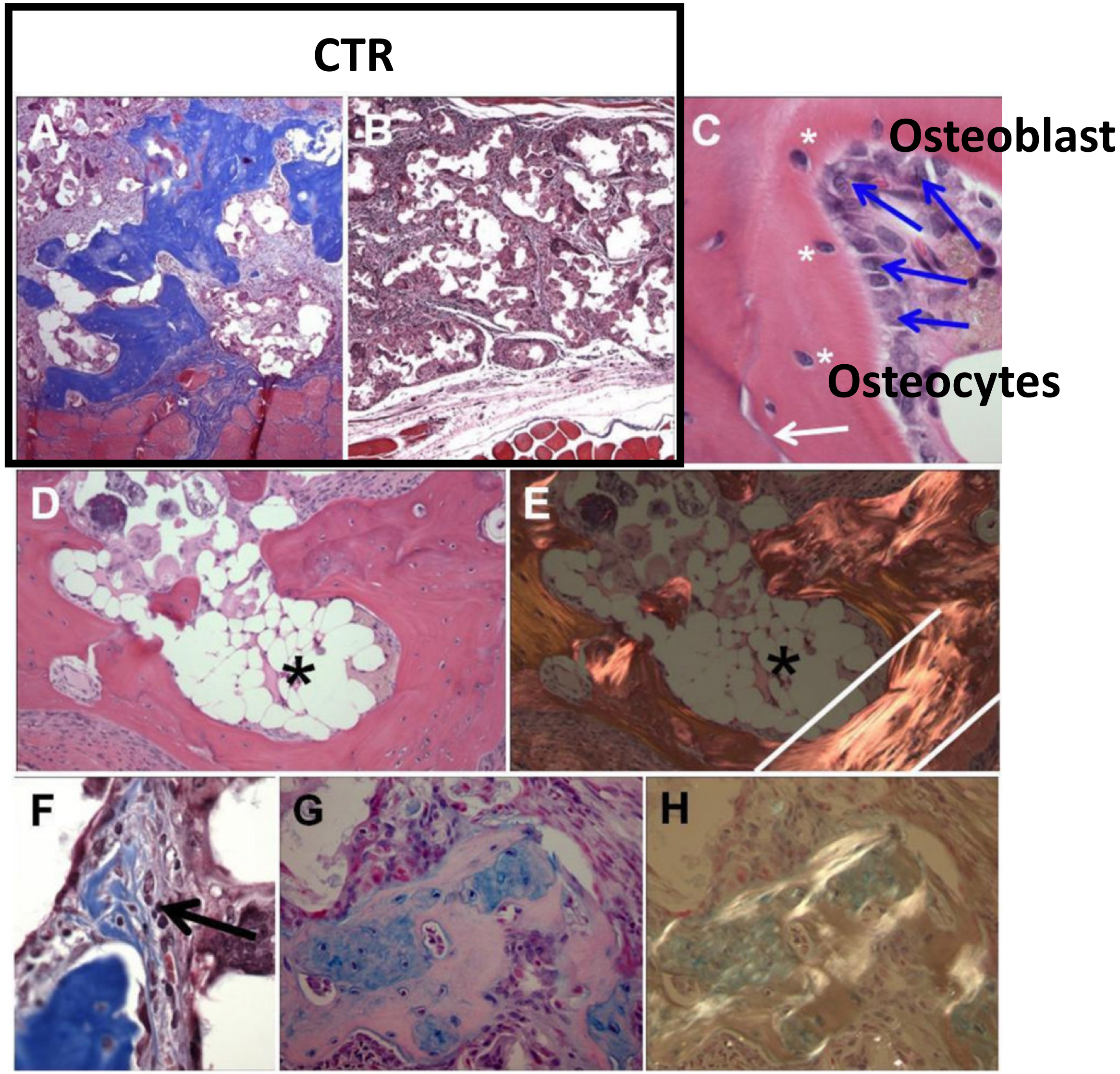


Sahoo et al., J Biomed Mater Res A. 2010 Jun 15;93(4):1539-50.
doi: 10.1002/jbm.a.32645.

Layer-by-Layer Self Assembly



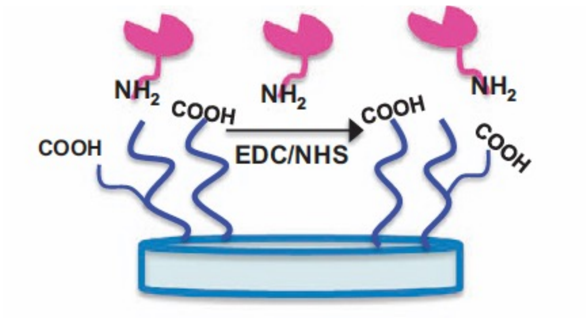




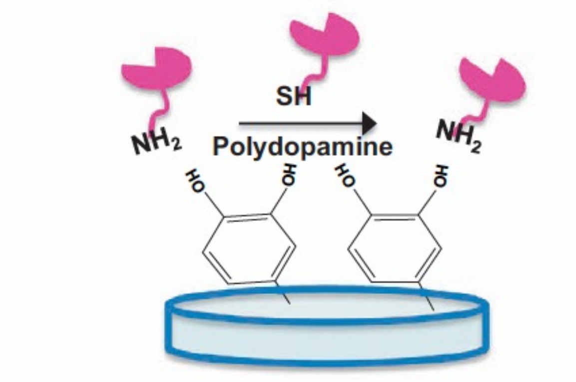
Scaffold: PCL/Tricalcium phosphate
1: Poly amino ester (+)
2: Chrondroitin sulfate (-)
3: BMP-2 (+)
4: Chondroitin sulfate (-)

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

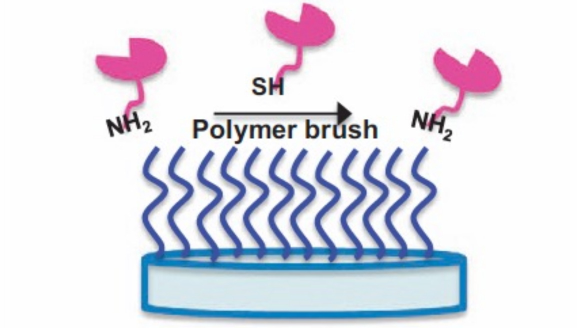
Covalent Conjugations



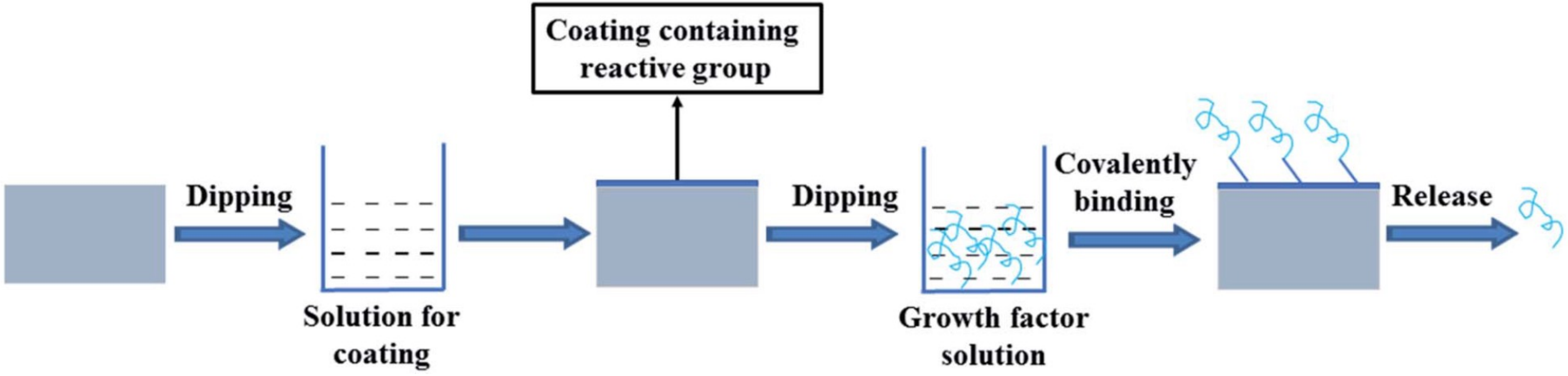
Carbodiimide coupling immobilization (EDC)

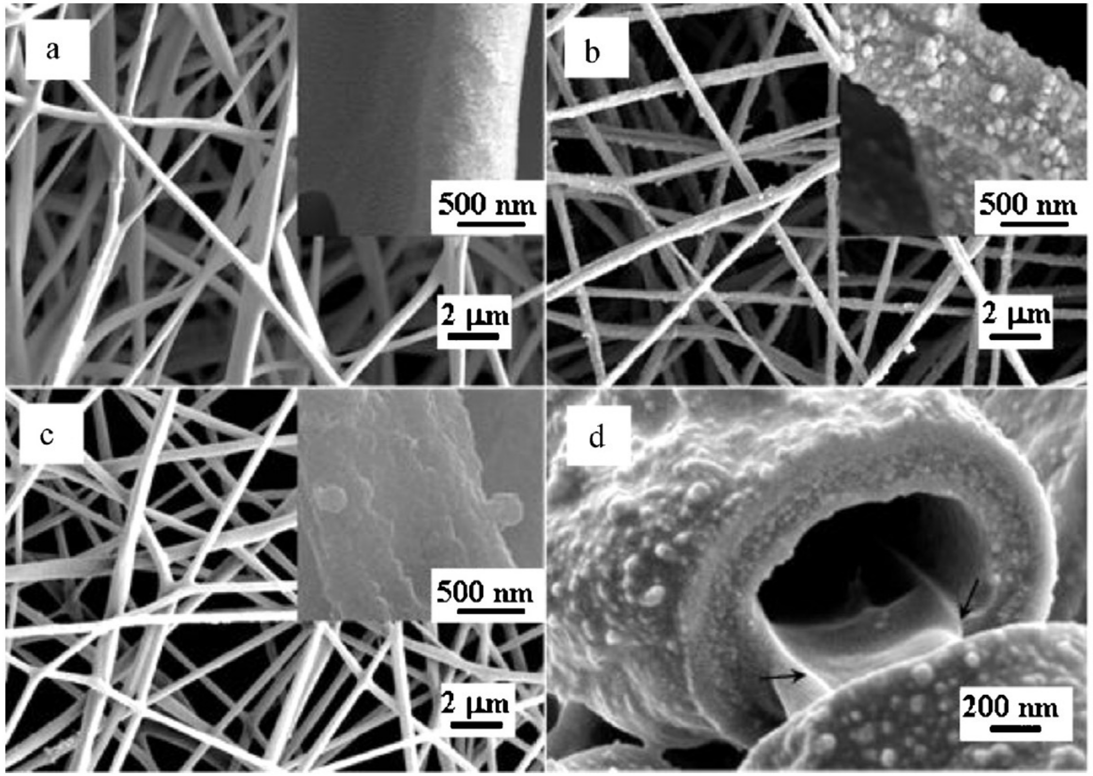


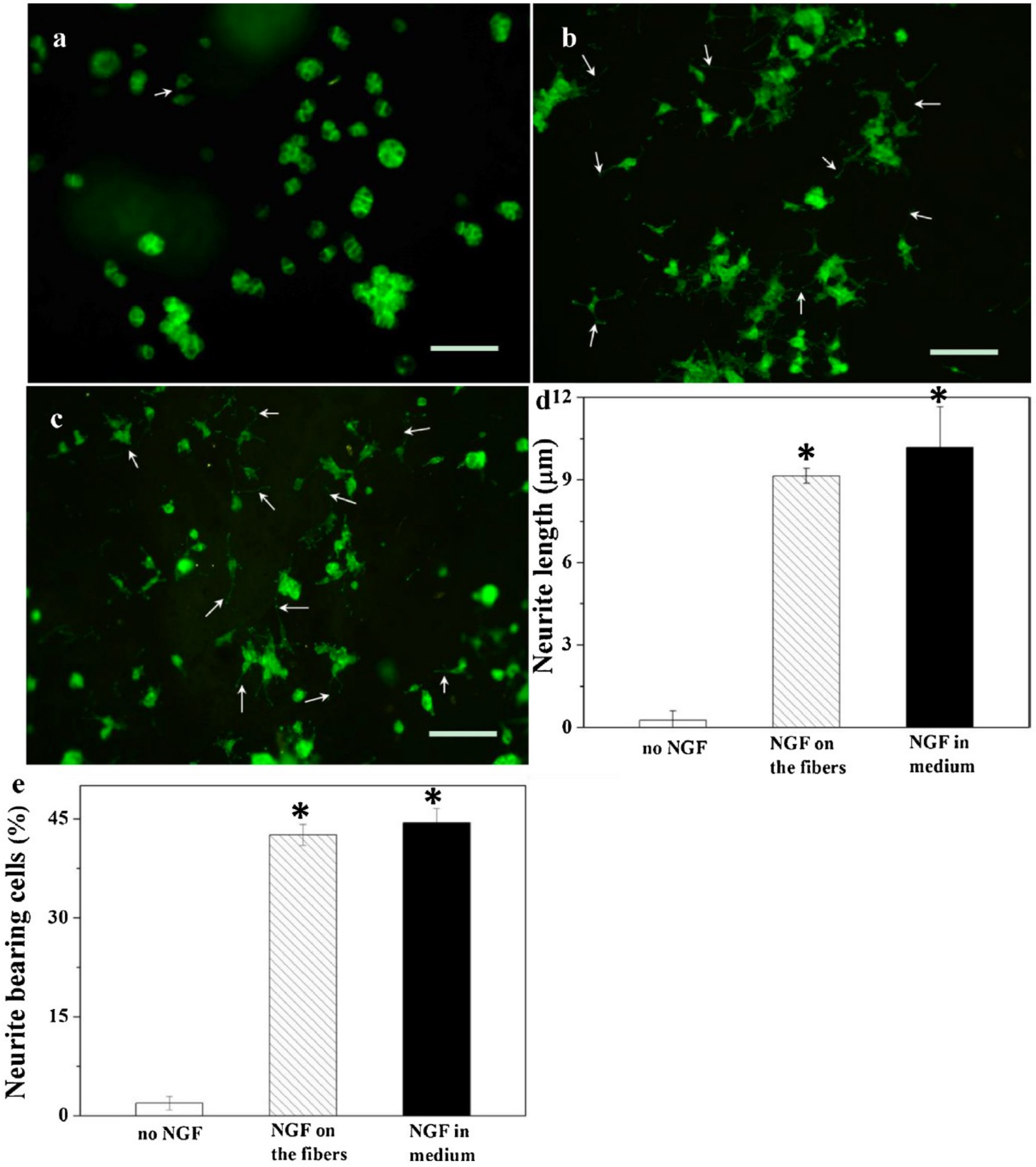
Mussel-inspired bioconjugations (PDA)



Other Chemical Coupling

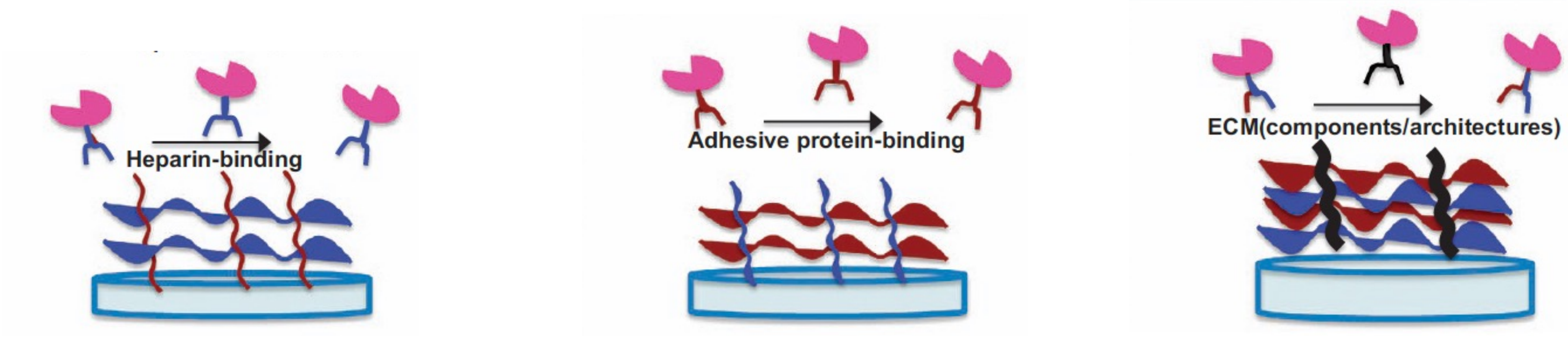


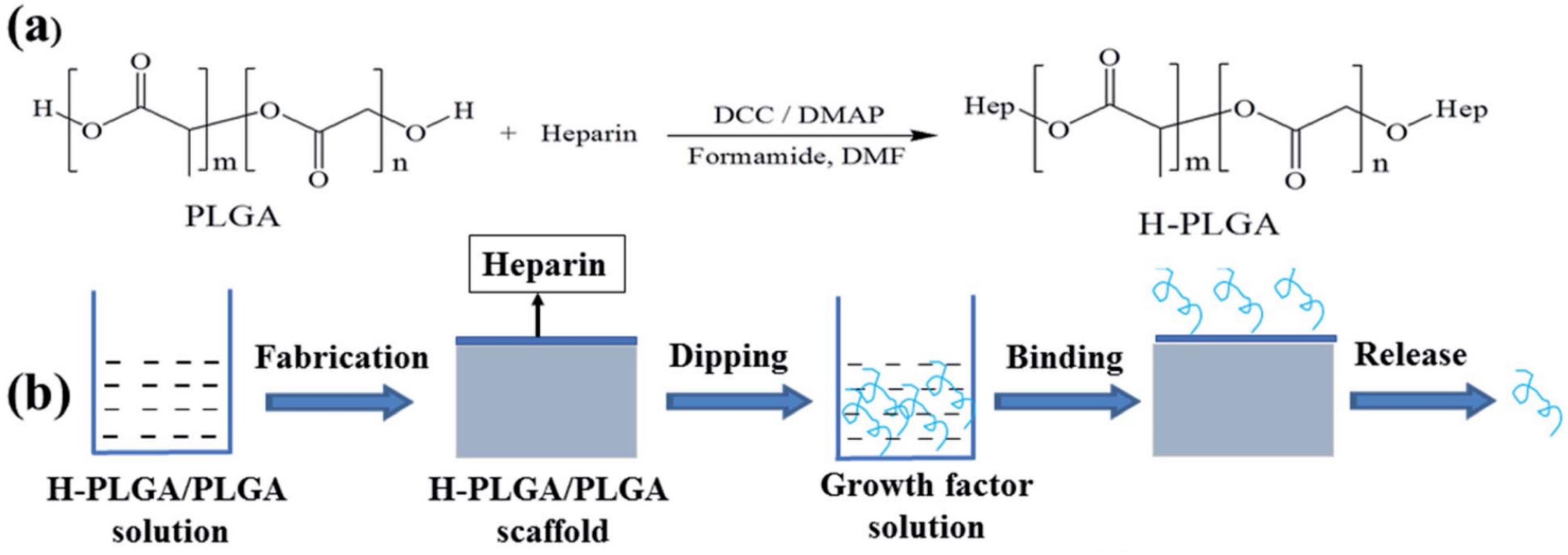




J. Zeng et al. / Colloids and Surfaces B: Biointerfaces *110 (2013) 450– 457*

ECM-Inspired Immobilization





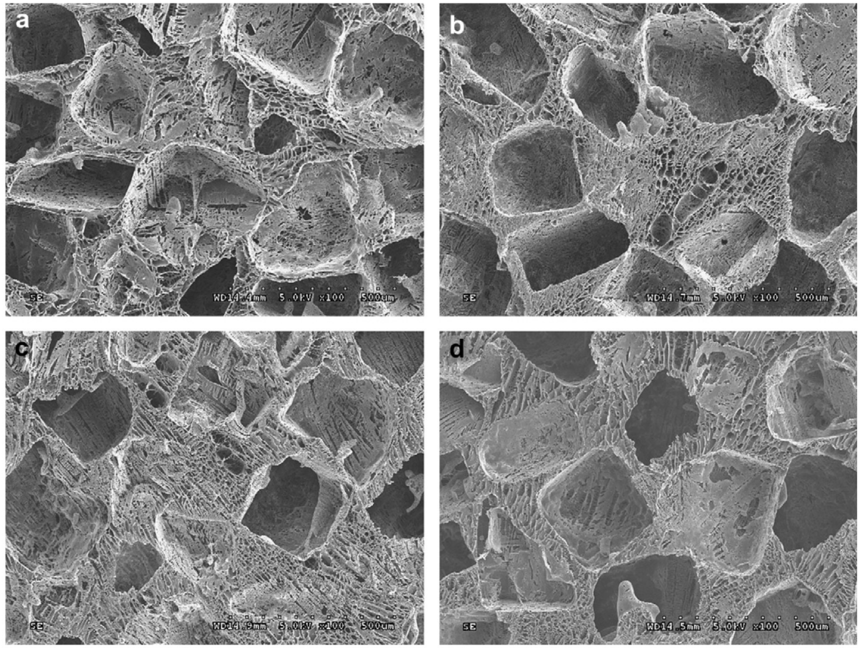
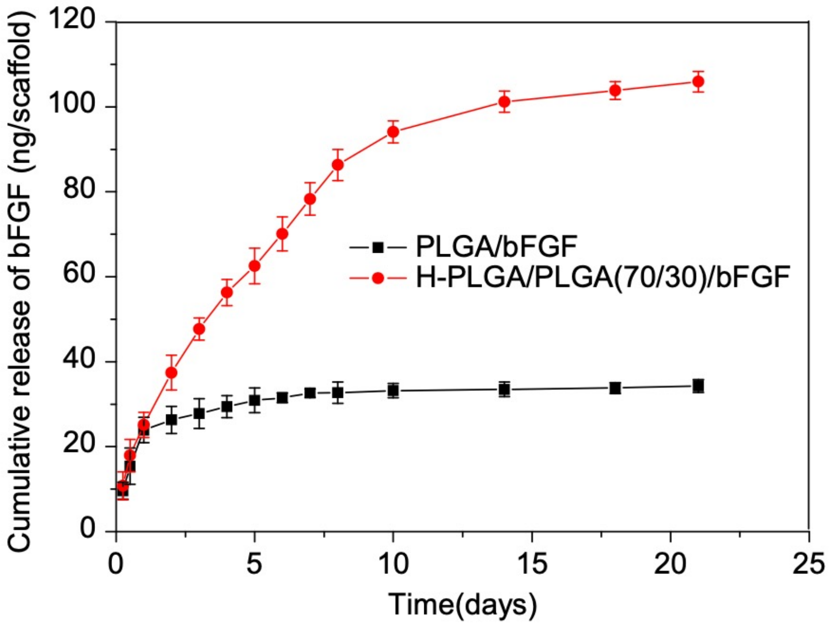
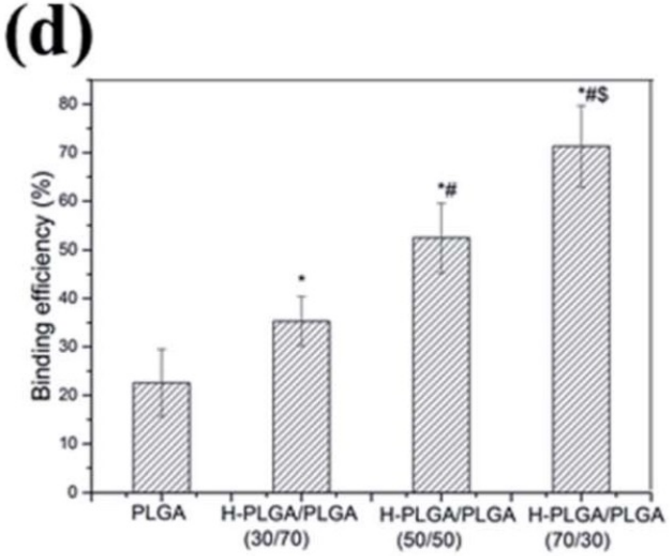
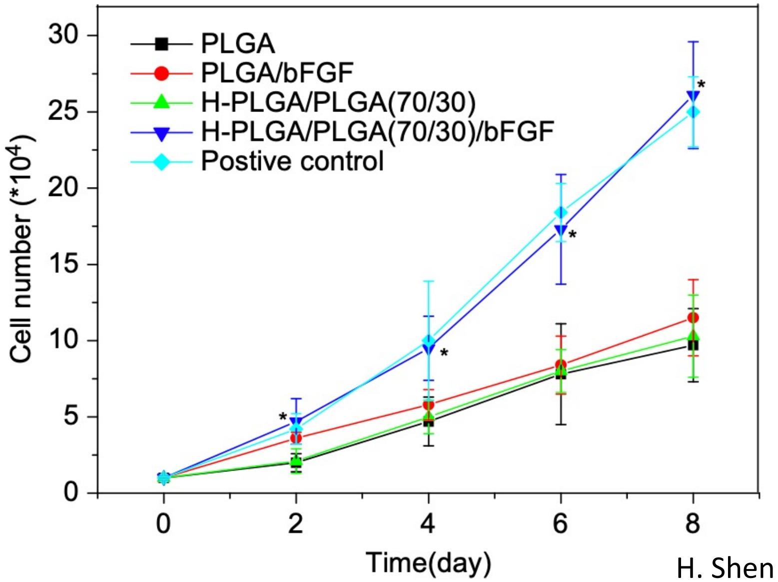


Fig. 2. Morphology structure of PLGA and H-PLGA/PLGA scaffolds. (a) PLGA; (b) H-PLGA/PLGA(30/70); (c) H-PLGA/PLGA(50/50); (d) H-PLGA/PLGA(70/30).

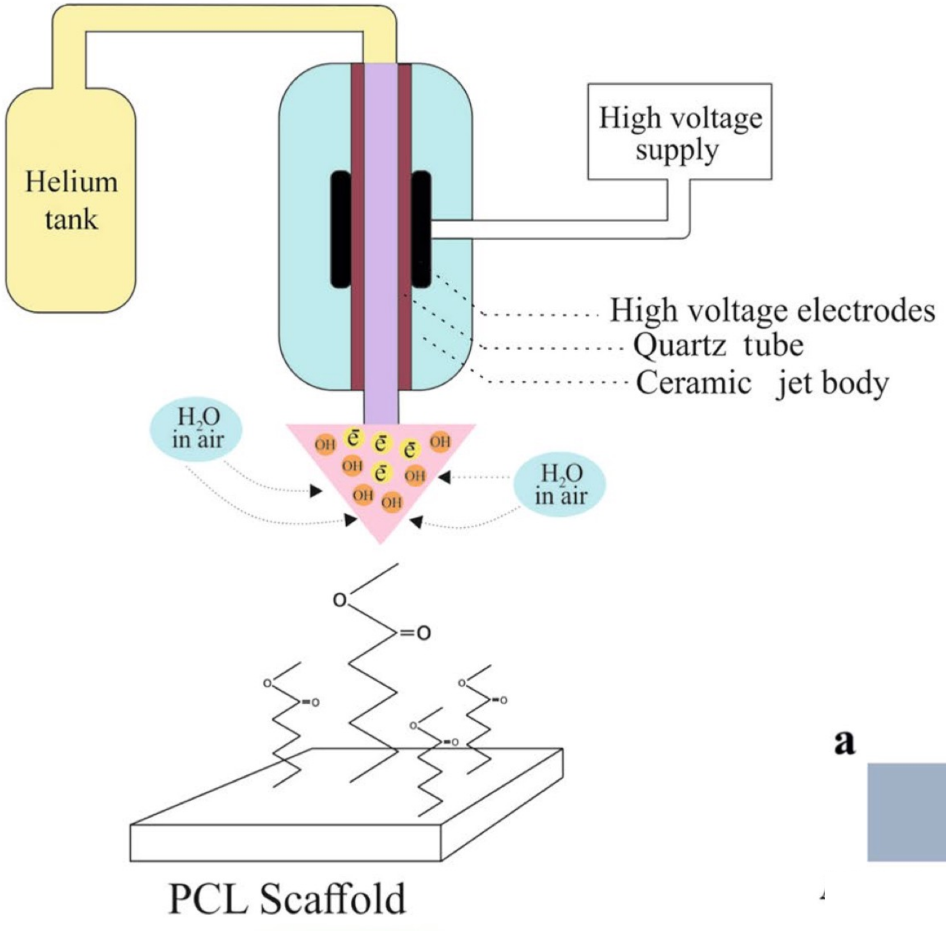


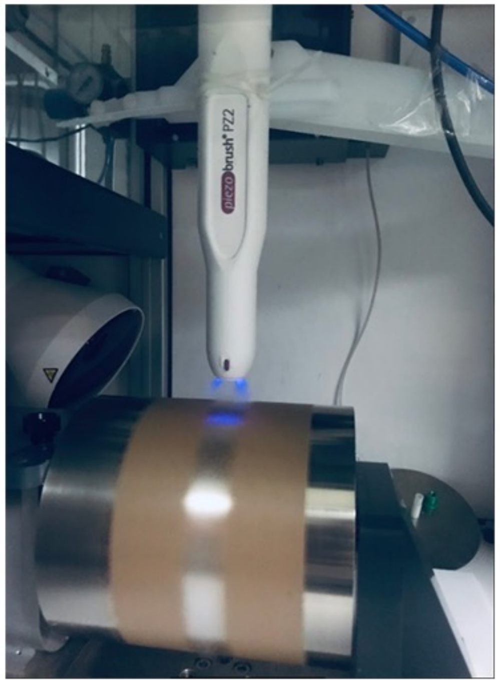




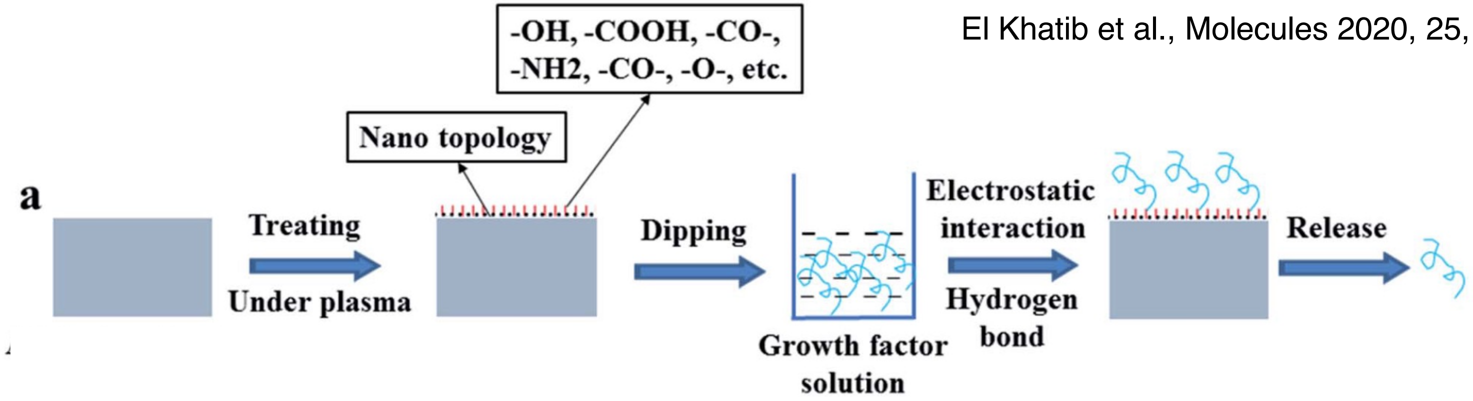
H. Shen et al. / Biomaterials 32 (2011) 3404e3412

Plasma Treatment

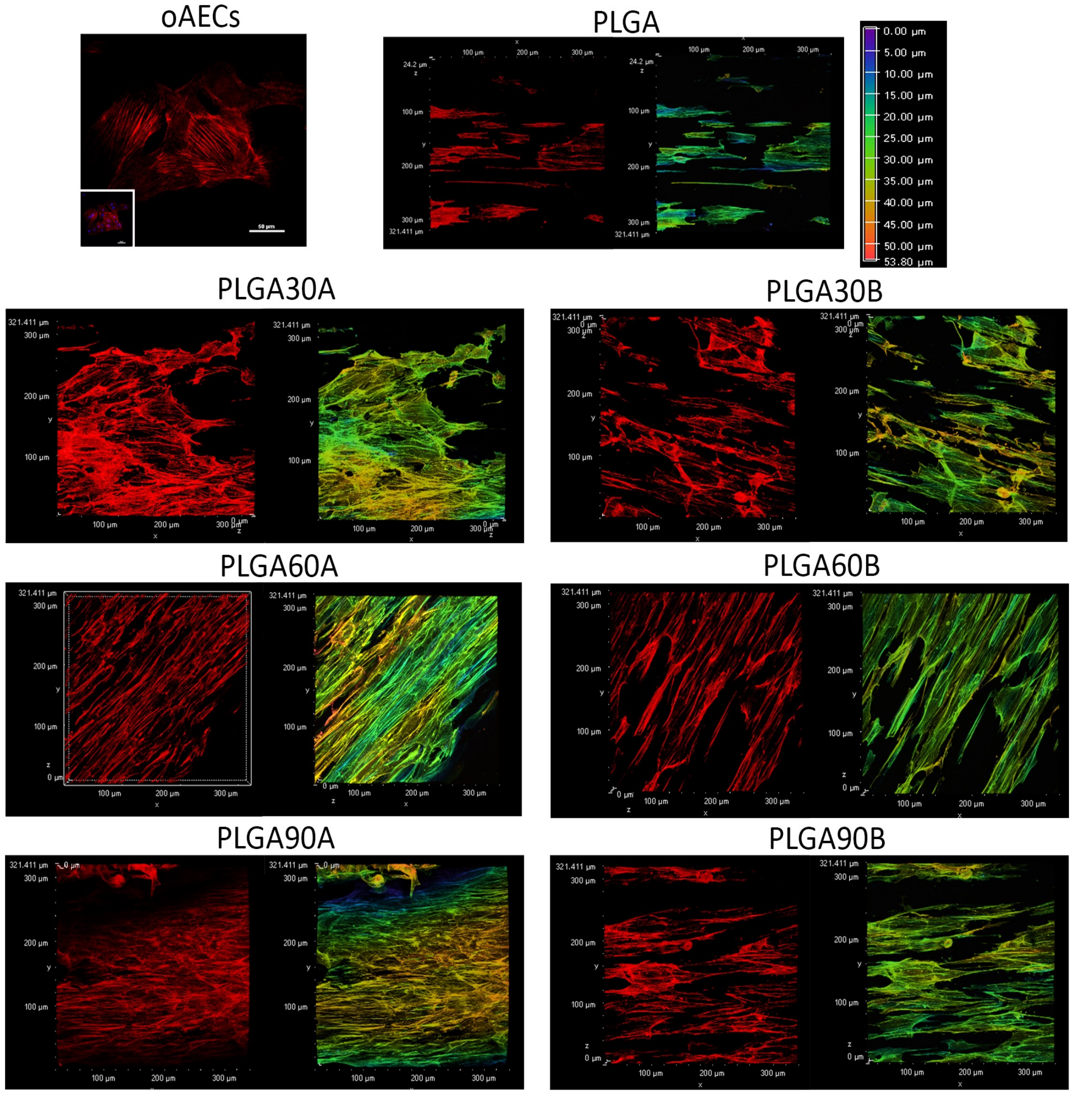




El Khatib et al., Molecules 2020, 25, 3176

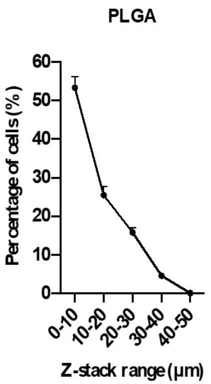


Meghdadi et al., Progress in Biomaterials (2019) 8:65–75

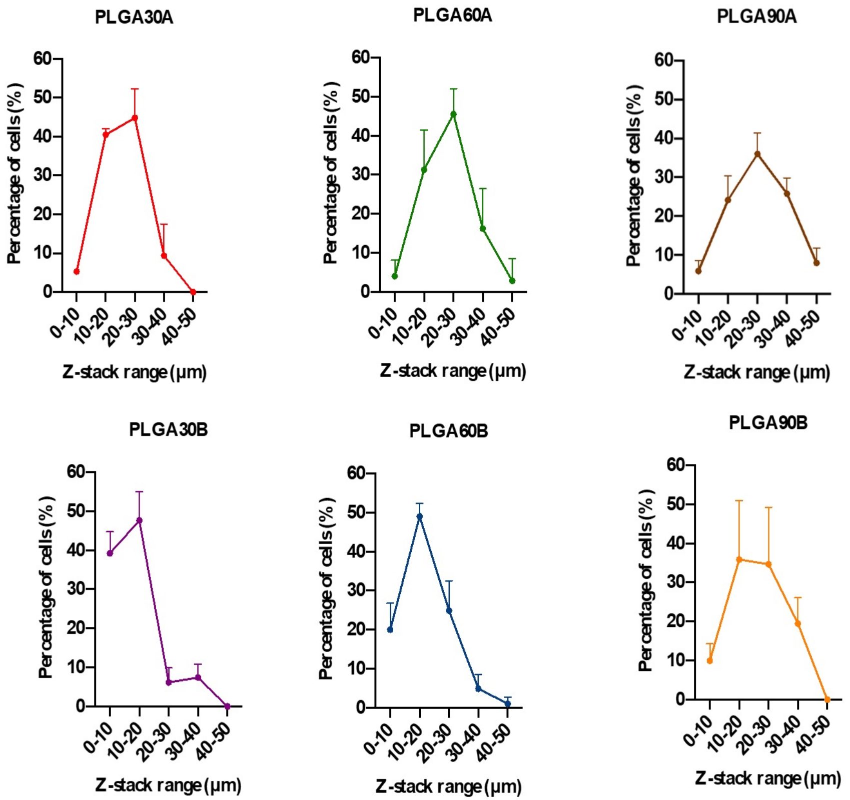


depth coded Maximum Intensity Projection (MaxIP).

Non-Treated



Treated with Cold Atmospheric Plasma



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

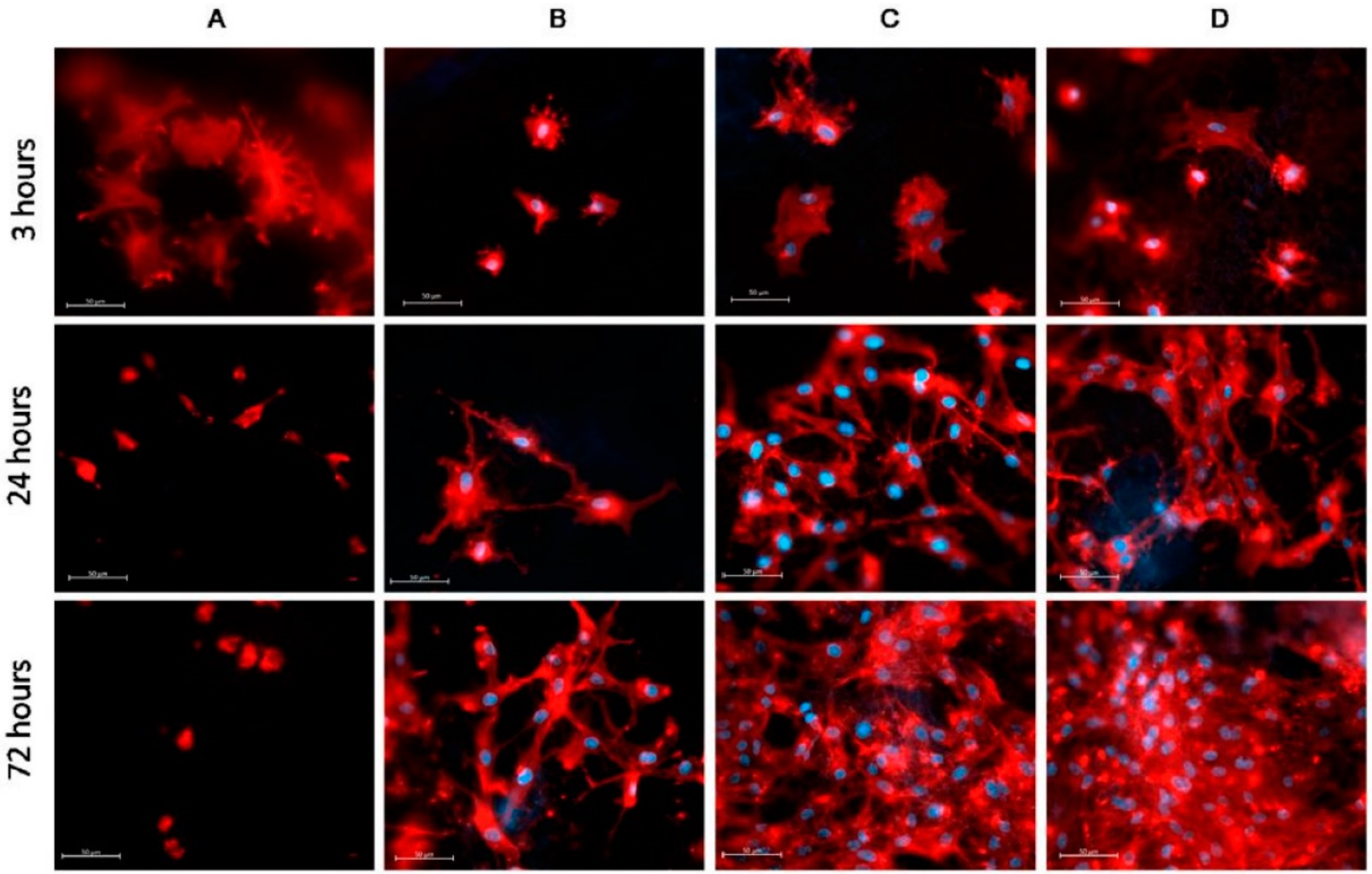
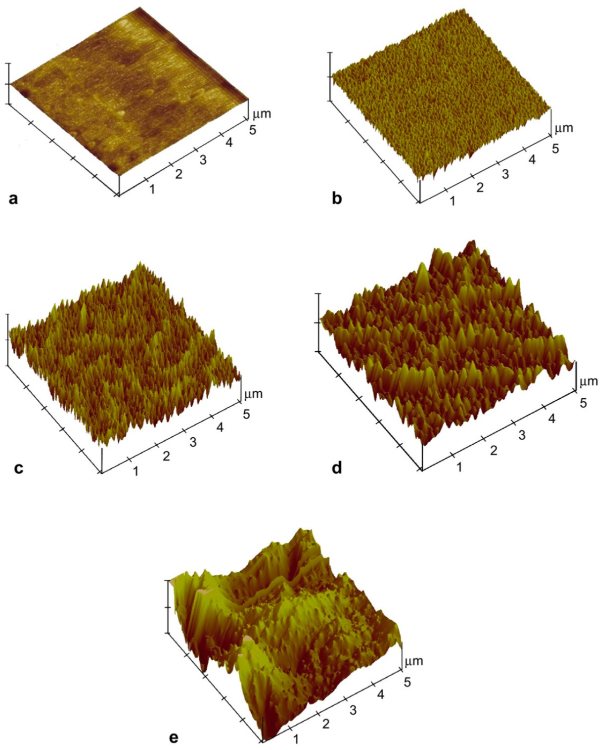
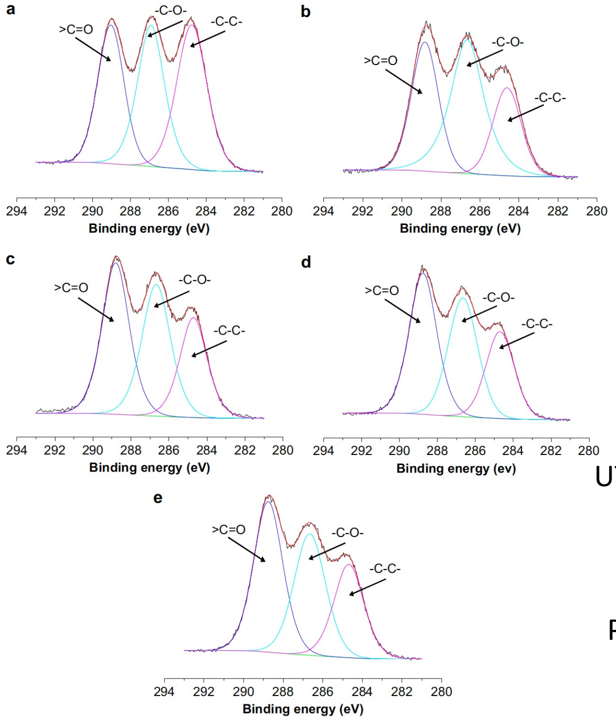


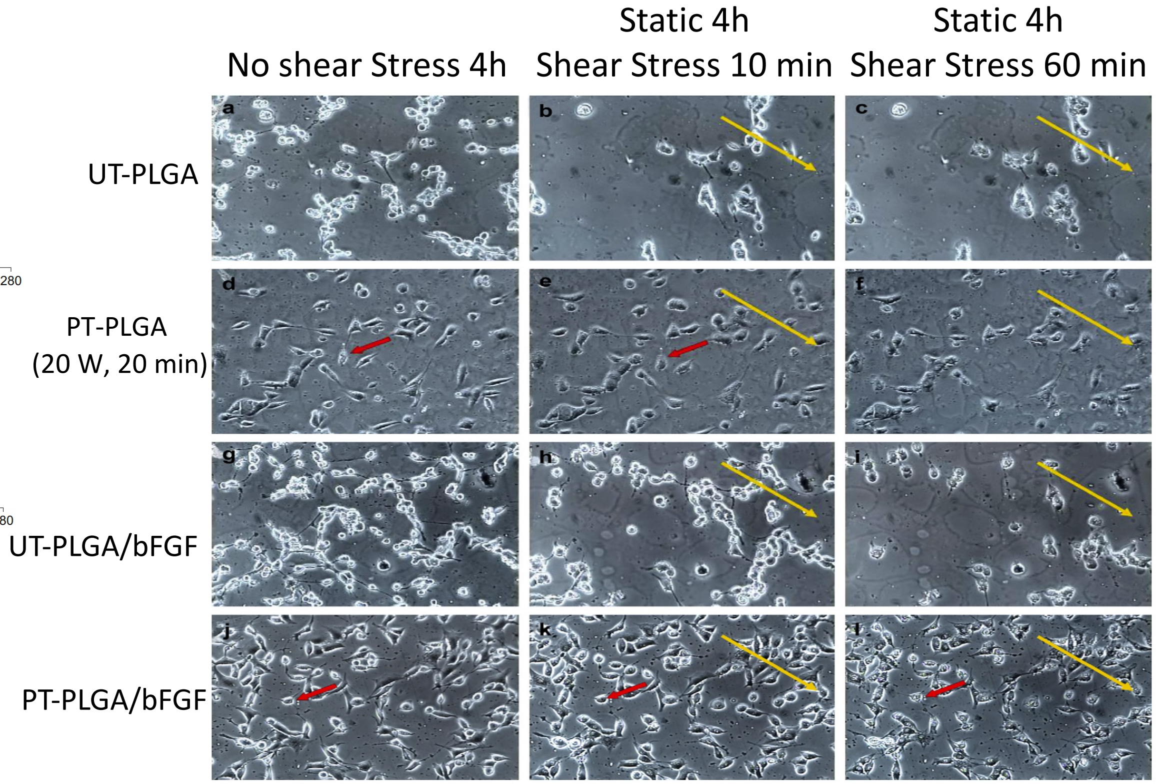
Figure 24. Adhesion of MSCs on the surface of untreated PCL (**A**), COOH-coated PCL (**B**), COOH-coated PCL with physically adsorbed PRP (**C**) and COOH-coated PCL with covalently immobilized PRP (**D**). All images were taken with a magnification of 40× and the scale bar corresponds to 50 µm—reproduced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

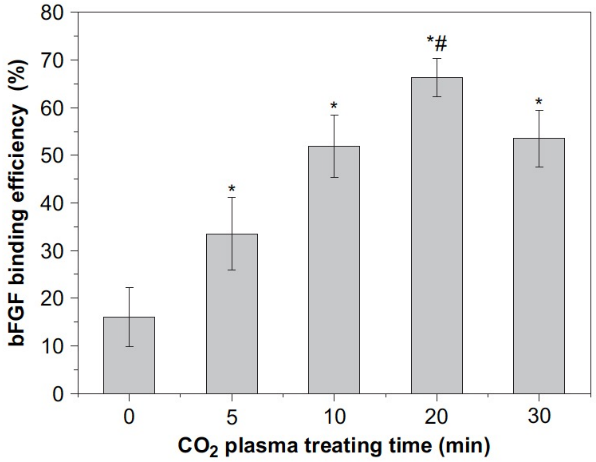
Asadian, Nanomaterials 2020, 10, 119; doi:10.3390/nano10010119

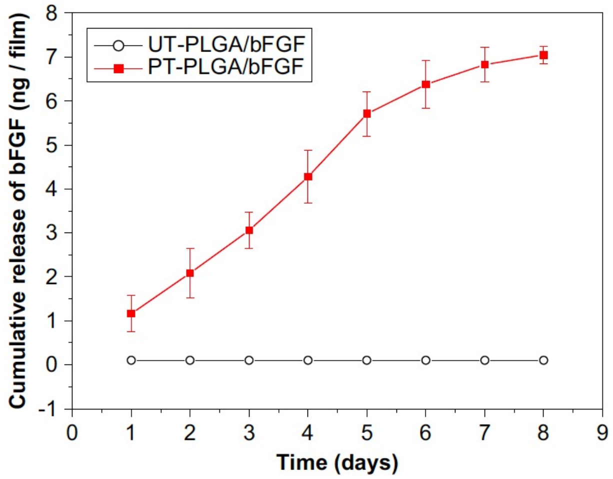
Treated with CO_2

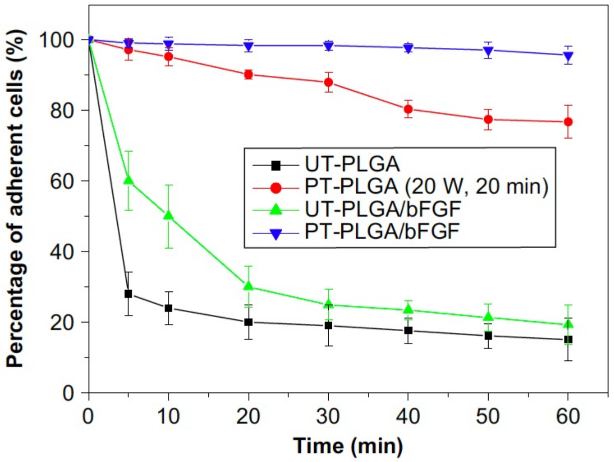






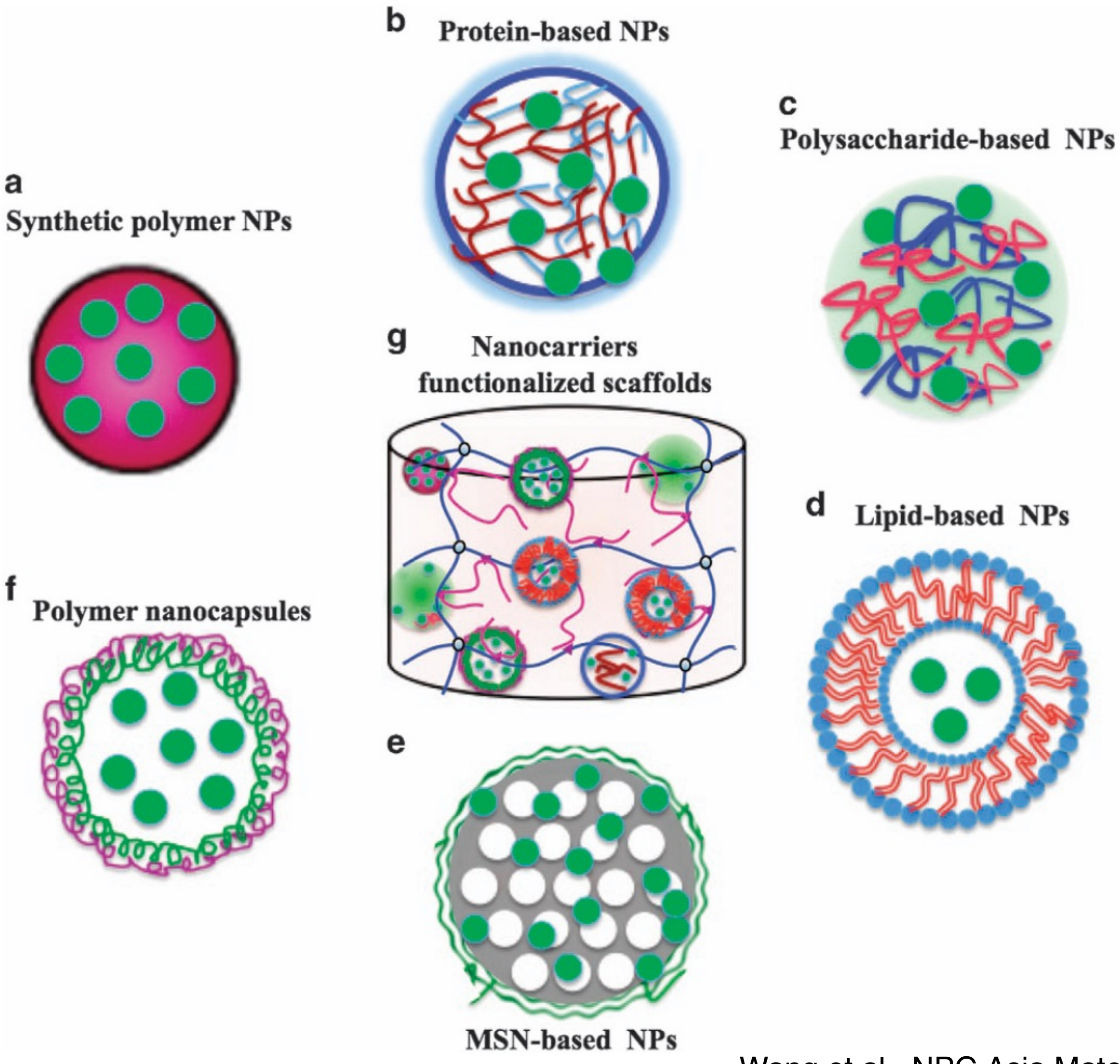




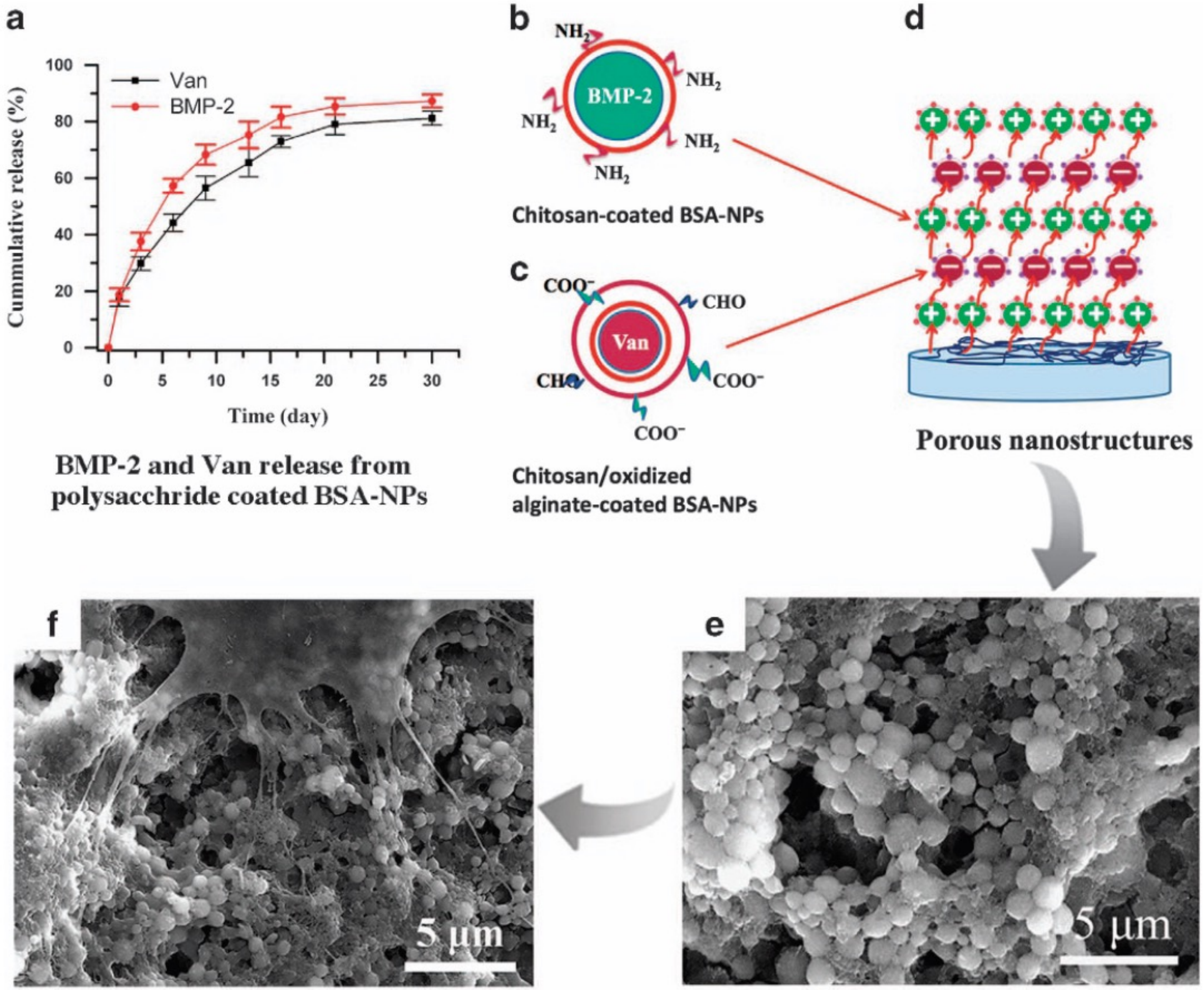


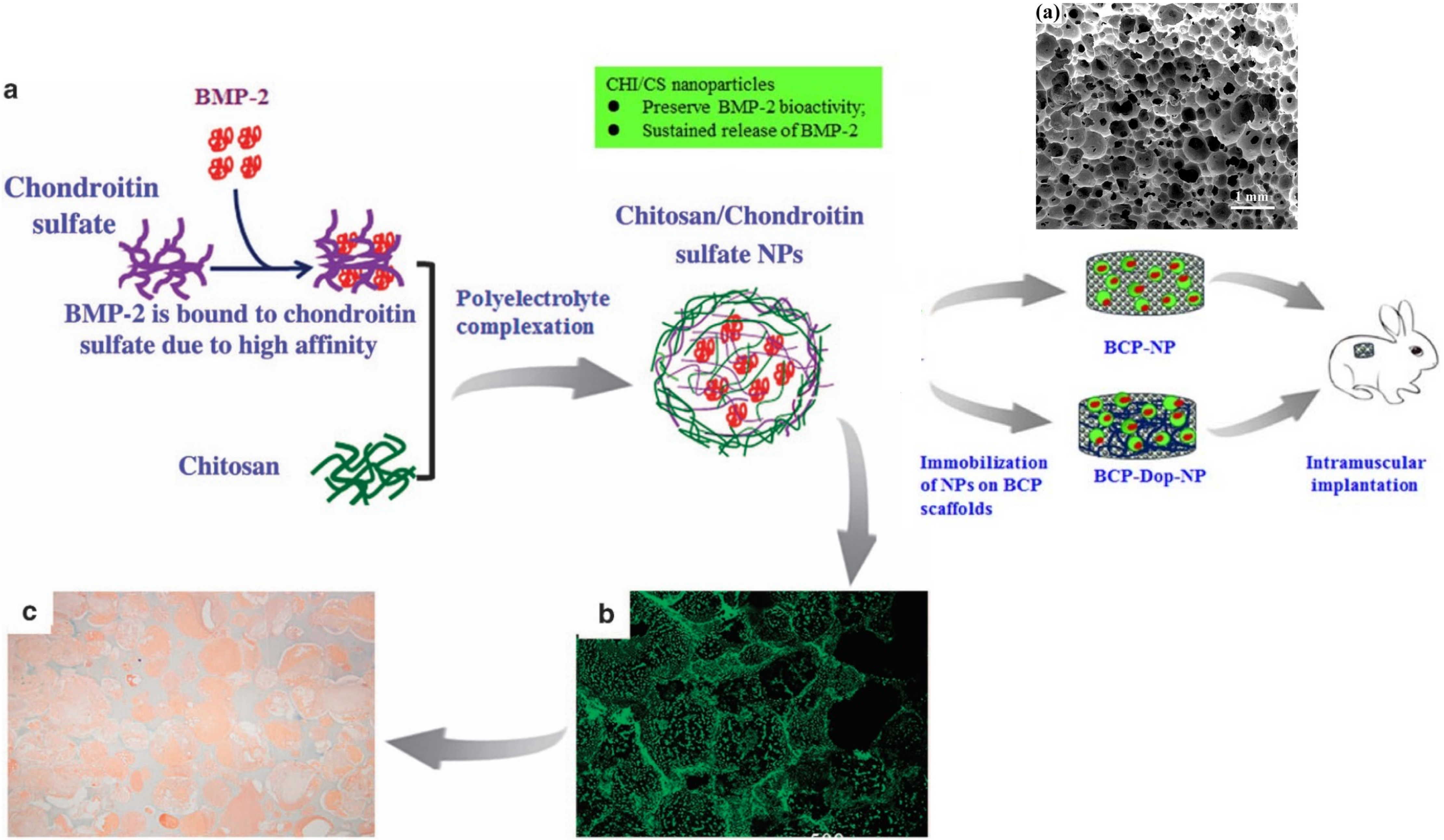
H. Shen et al. / Biomaterials 29 (2008) 2388e2399, doi:10.1016/j.biomaterials.2008.02.008

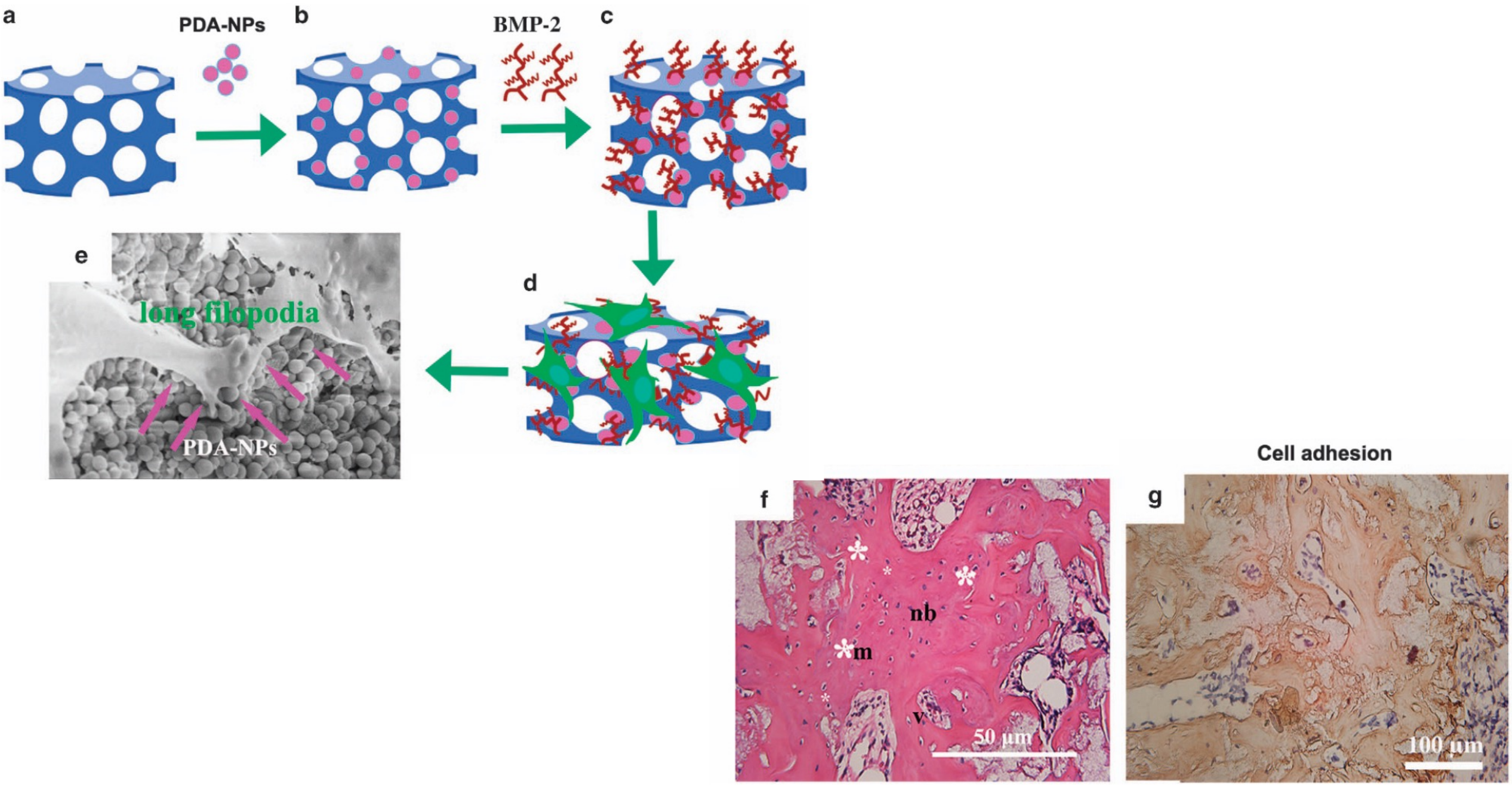
Nanocarriers for GF Encapsulation and release for Biomedical Applications



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


a
Van
BMP-2
Cummulative release (%)
Time (day)
BMP-2 and Van release from polysacchride coated BSA-NPs
b
NH2
BMP-2
Chitosan-coated BSA-NPs
c
COO⁻
CHO
Van
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

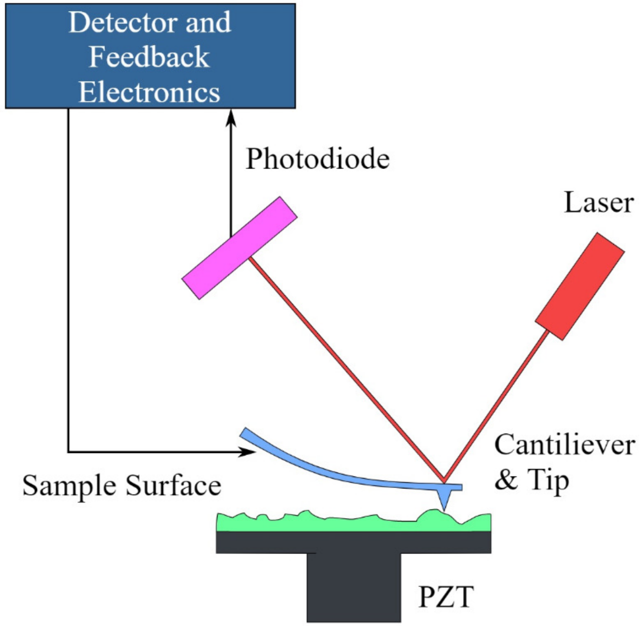

a
PDA-NPs
b
BMP-2
c
d
e
long filopodia
PDA-NPs
Cell adhesion
f
nb
m
v
50 µm
g
100 µm

Techniques for the physicochemical analysis of the surface functionalization

- Microscopy Techniques
- Surface Wettability
- Colorimetric Analysis
- Spectro-scopy Techniques

Microscopy Techniques

Atomic Force Microscopy (AFM)



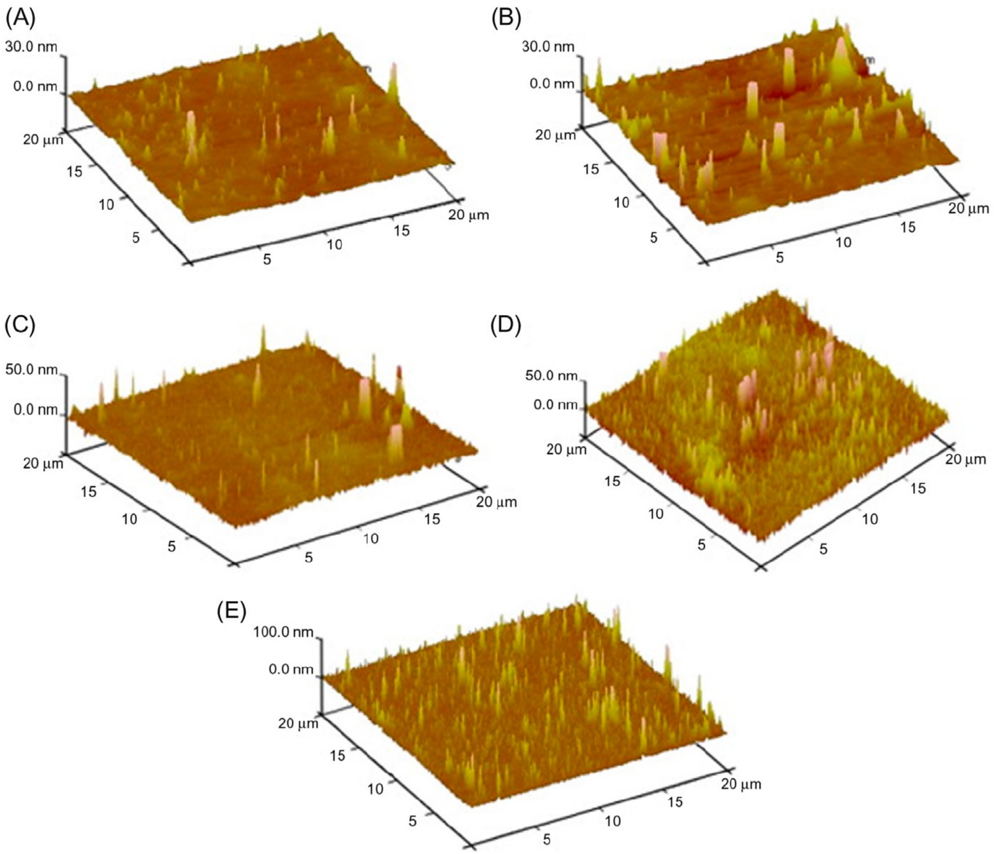


Figure 11.7 AFM topographic of (A) gelatin substrate and gelatin substrates with (B) 1, (C) 6, (D) 9, and (E) 10 layers.

Microscopy Techniques

Scanning Electron Microscopy (SEM)

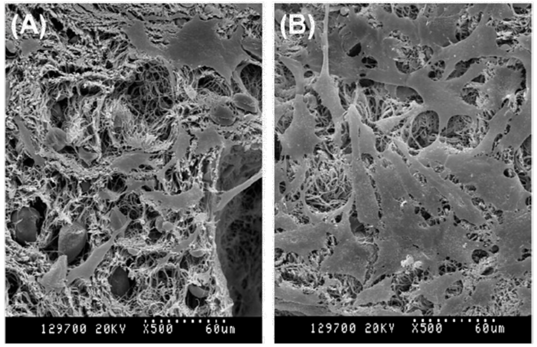
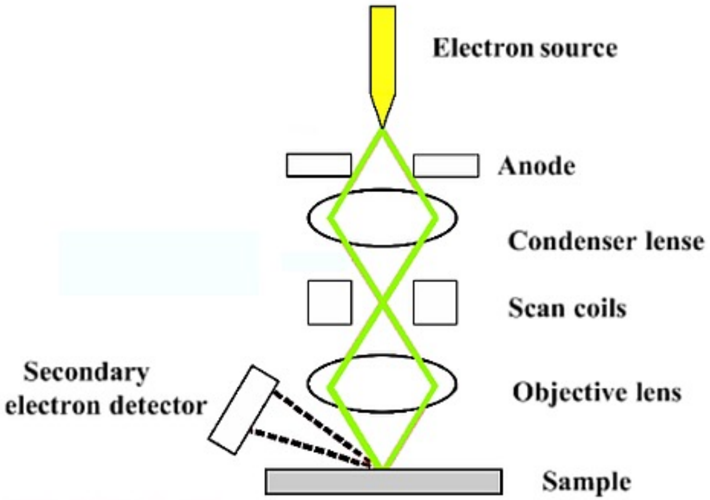
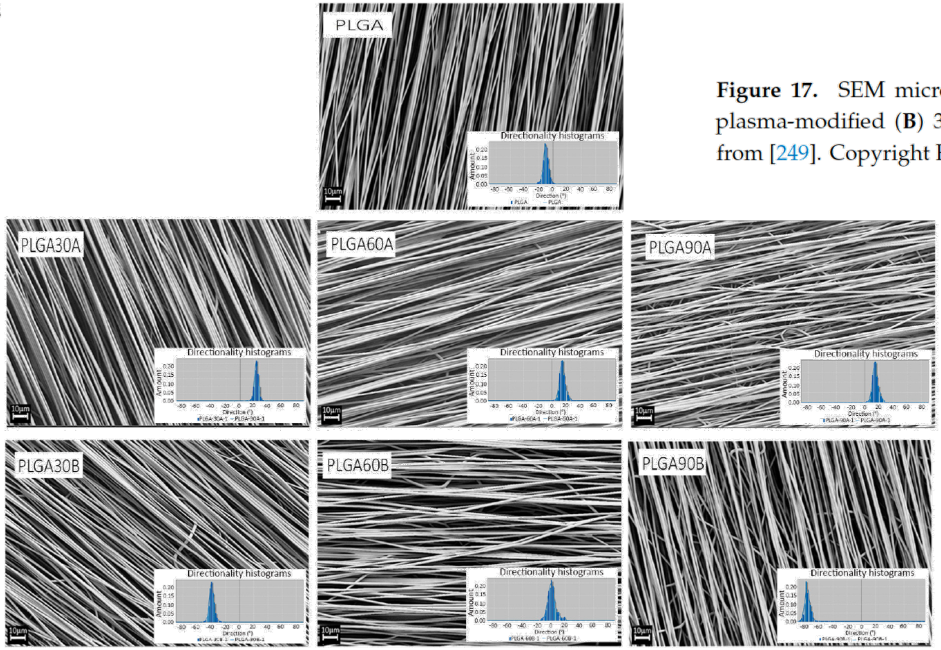


Figure 17. SEM micrographs of nHAC-kn cultured for seven days onto untreated (**A**) and Ar plasma-modified (**B**) 3D porous nanofibrous silk fibroin scaffolds—reproduced with permission from [249]. Copyright Elsevier, 2008.

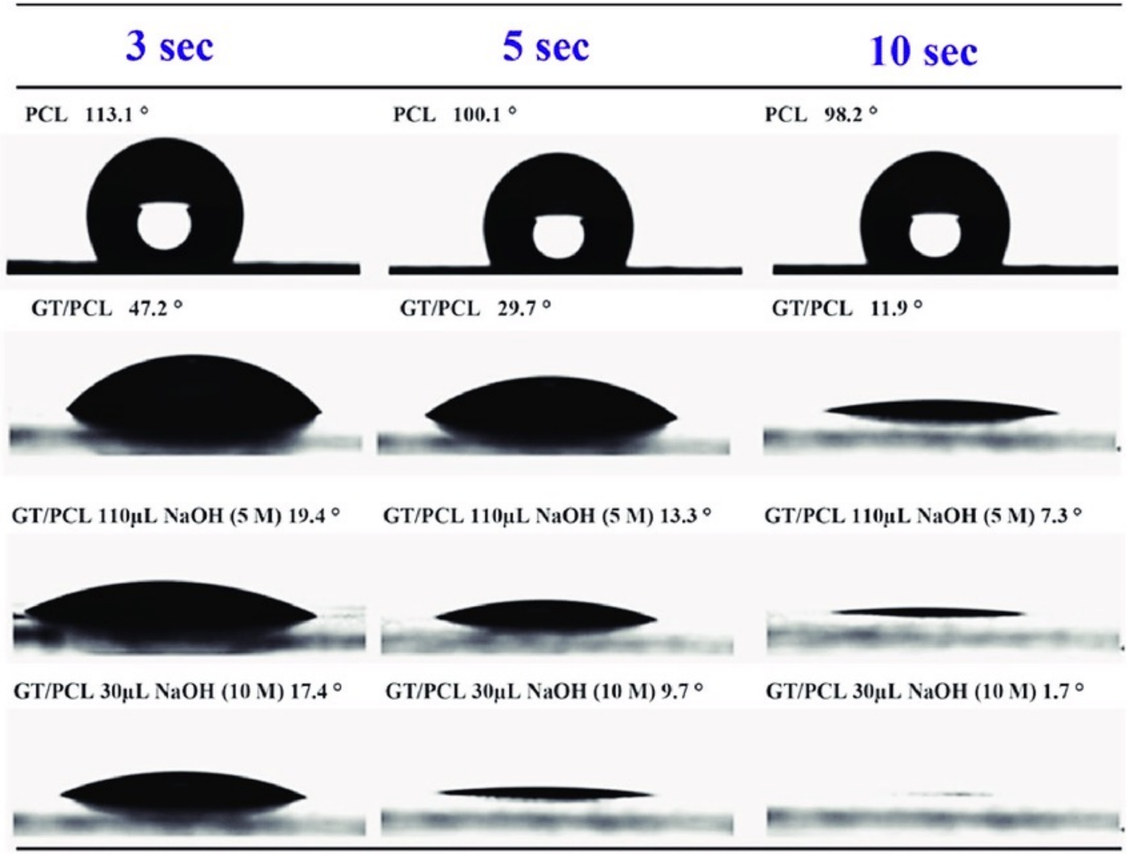
Scanning Electron Microscope



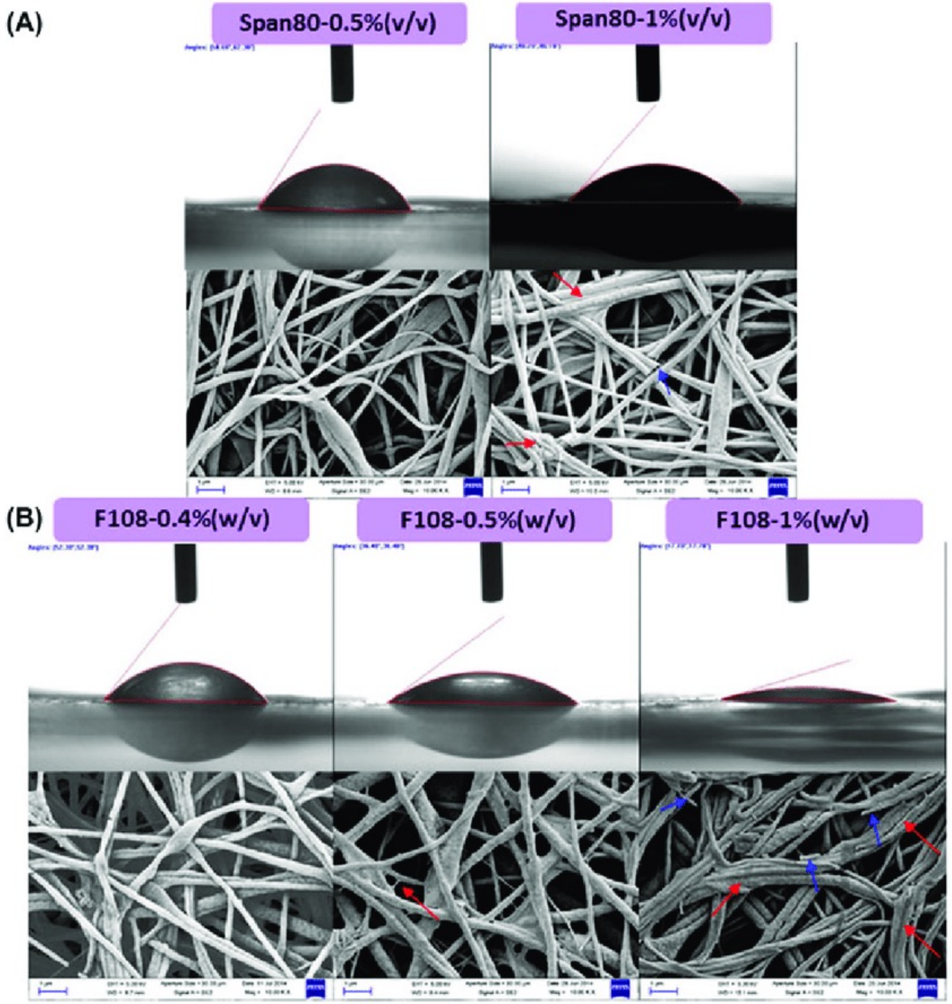


El Khatib et al., Molecules 2020, 25, 3176

Surface Wettability

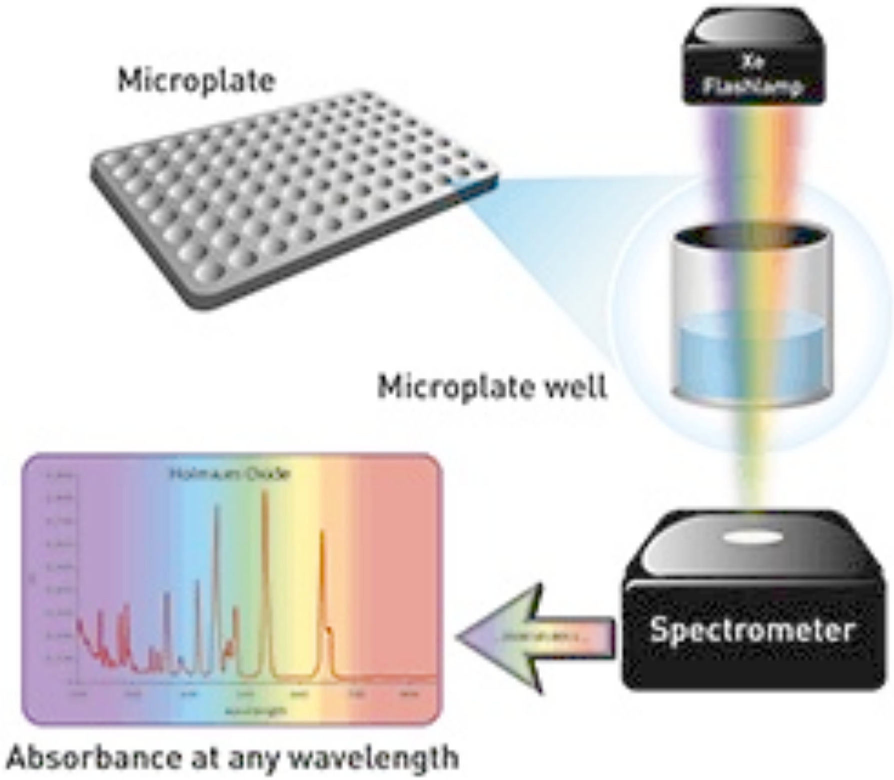


Zhou et al., Macromol. Biosci. 2017, 1700268

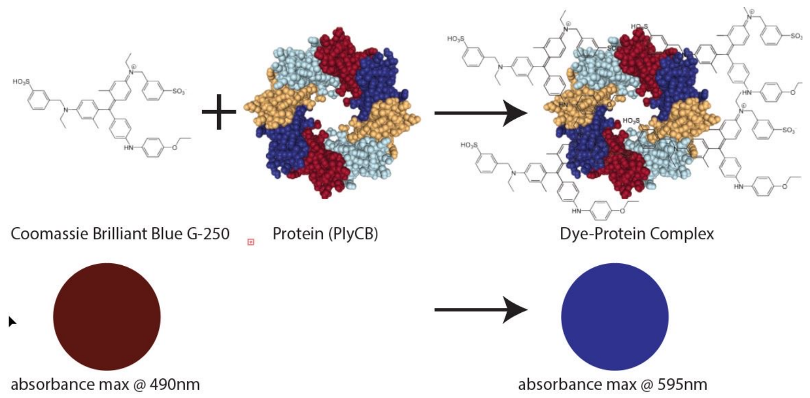


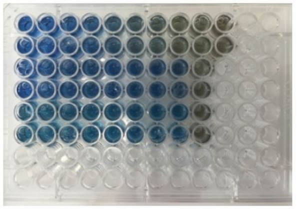
Jue Hu, (2015), Journal of Biomaterial Science, Polymer Edition, 26:1; 57-75

Colorimetric analysis

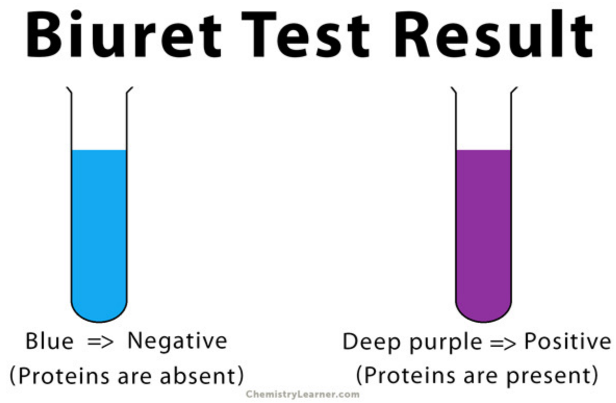


Bradford assay



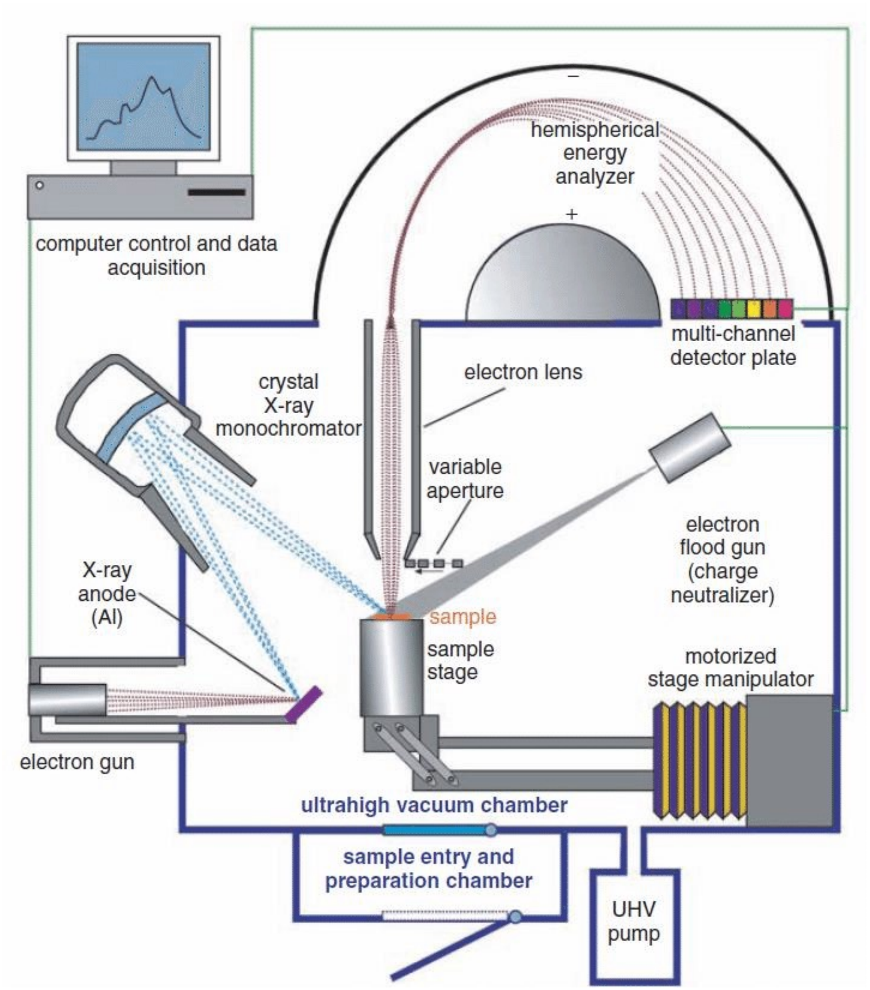


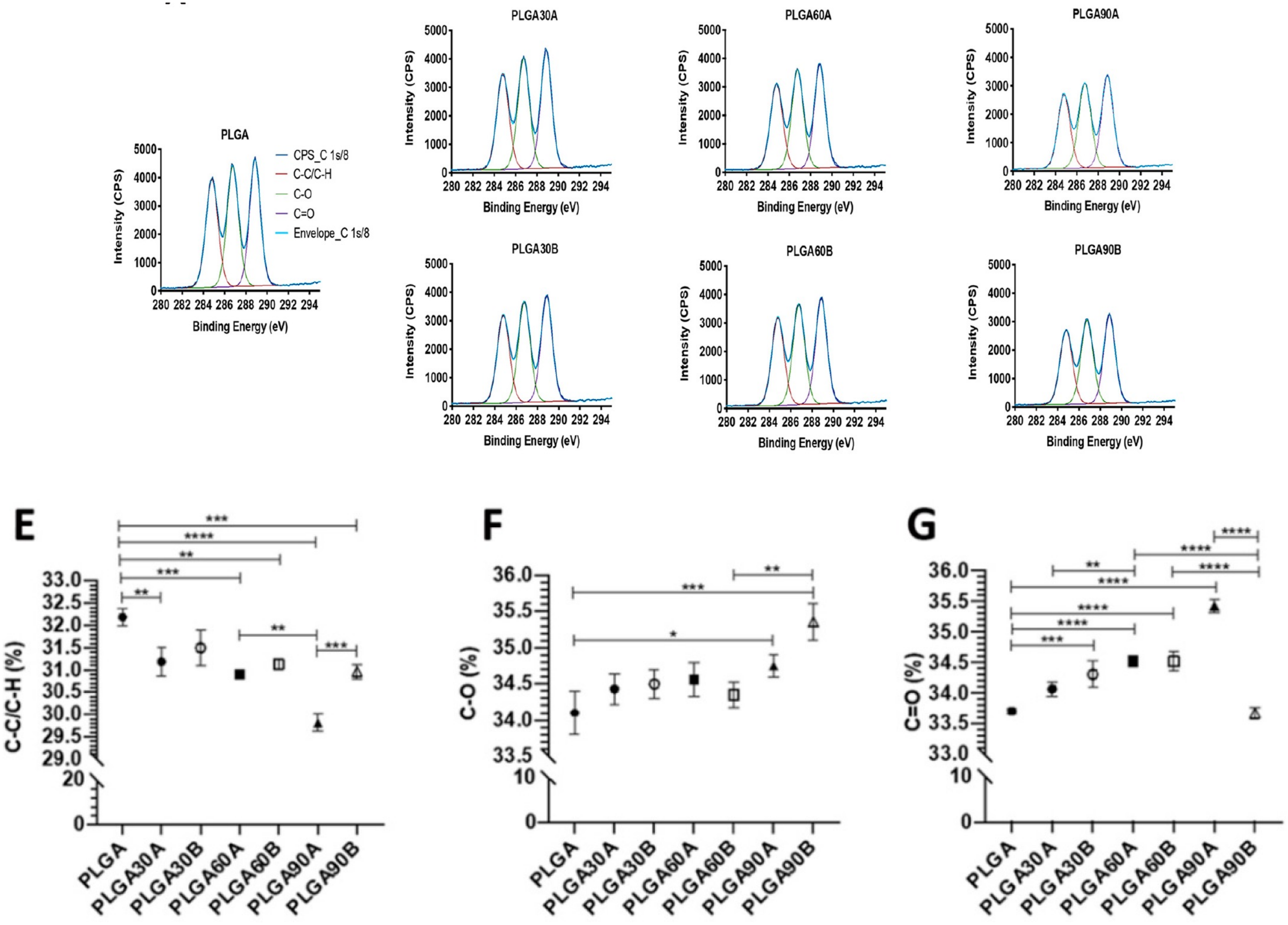
Bradford assay in 96-well plate containing wells with and without protein.



Spectroscopy Techniques

XPS analysis





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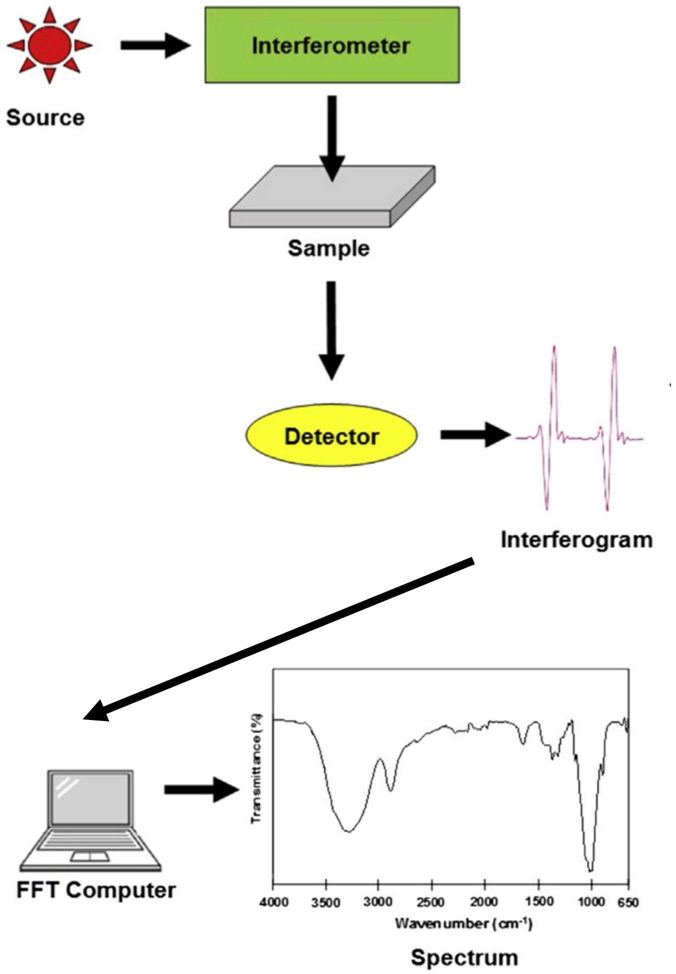
Spectroscopy Techniques

FTIR Spectroscopy



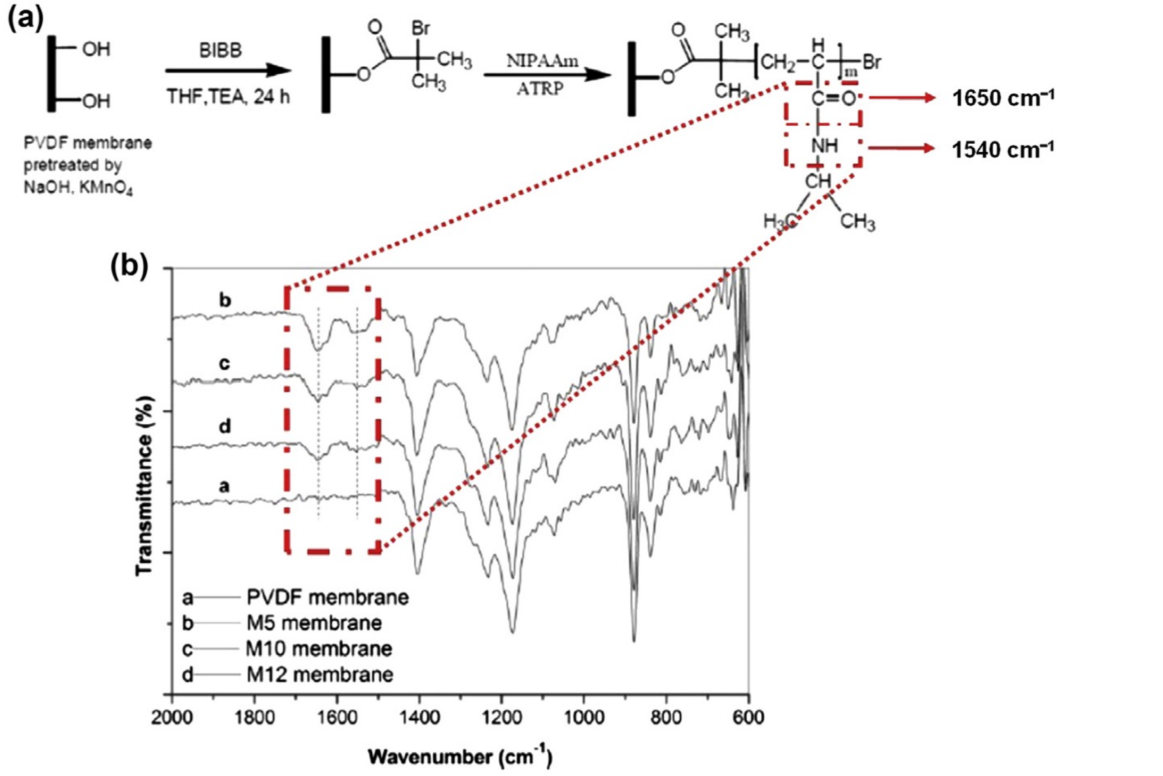


Figure 1.10

(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.*