Extracellular Matrix Analogues for the Pre-Clinical Handling and Transplantation of Cells

ETP on Nanomedicine Webinar
4th October 2016

Matteo Santin
Brighton Studies in Tissue-mimicry and Aided Regeneration (*BrightSTAR*)
Brighton Centre for Regenerative Medicine
School of Pharmacy & Biomolecular Sciences

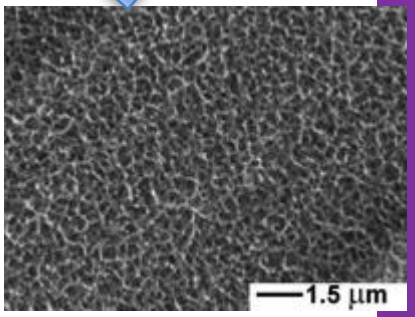
University of Brighton

A New Generation of Biomaterials: From Tissue Re-Placement to Tissue Re-generation

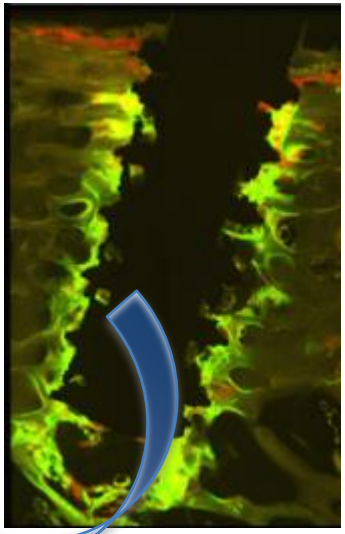
Tissue Replacement

Adverse Reactions

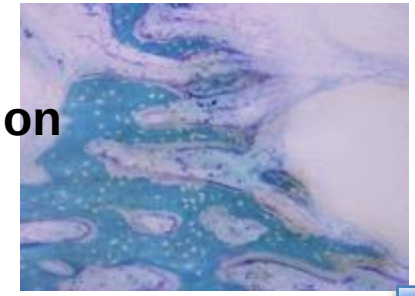
Surface Modification



Tissue Integration



Tissue Regeneration

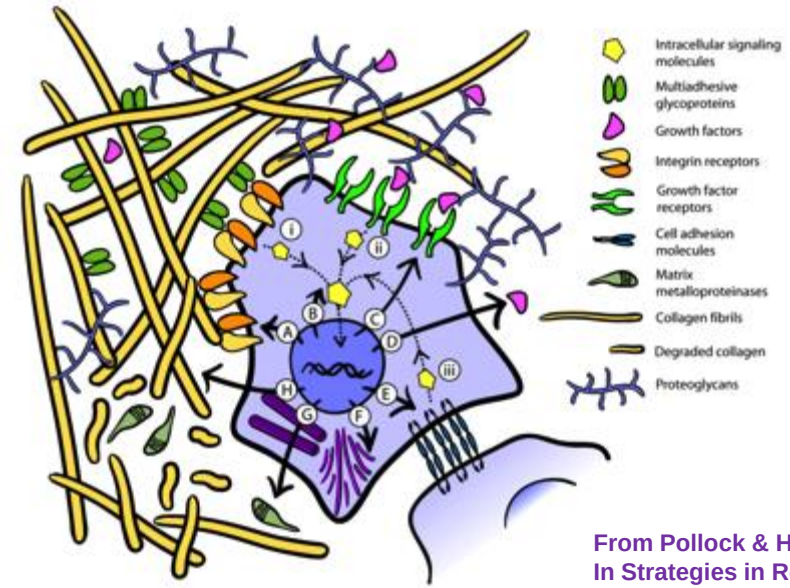


University of Brighton

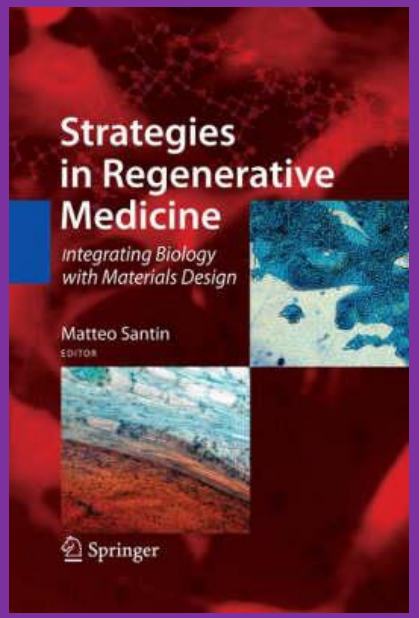
Tissue Microenvironment

- **Modern synthetic biomaterials** fully integrating molecular cues mimicking certain aspects of structure or function of natural extracellular microenvironments

MP Lutolf, JA Hubbell, 2005,
***Nat Biotechnol* 23 (1): 47-55**



From Pollock & Healy
In Strategies in Regenerative Medicine
M Santin ed, Springer 2009, Chapter 4



The Extracellular Matrix Analogue Concept

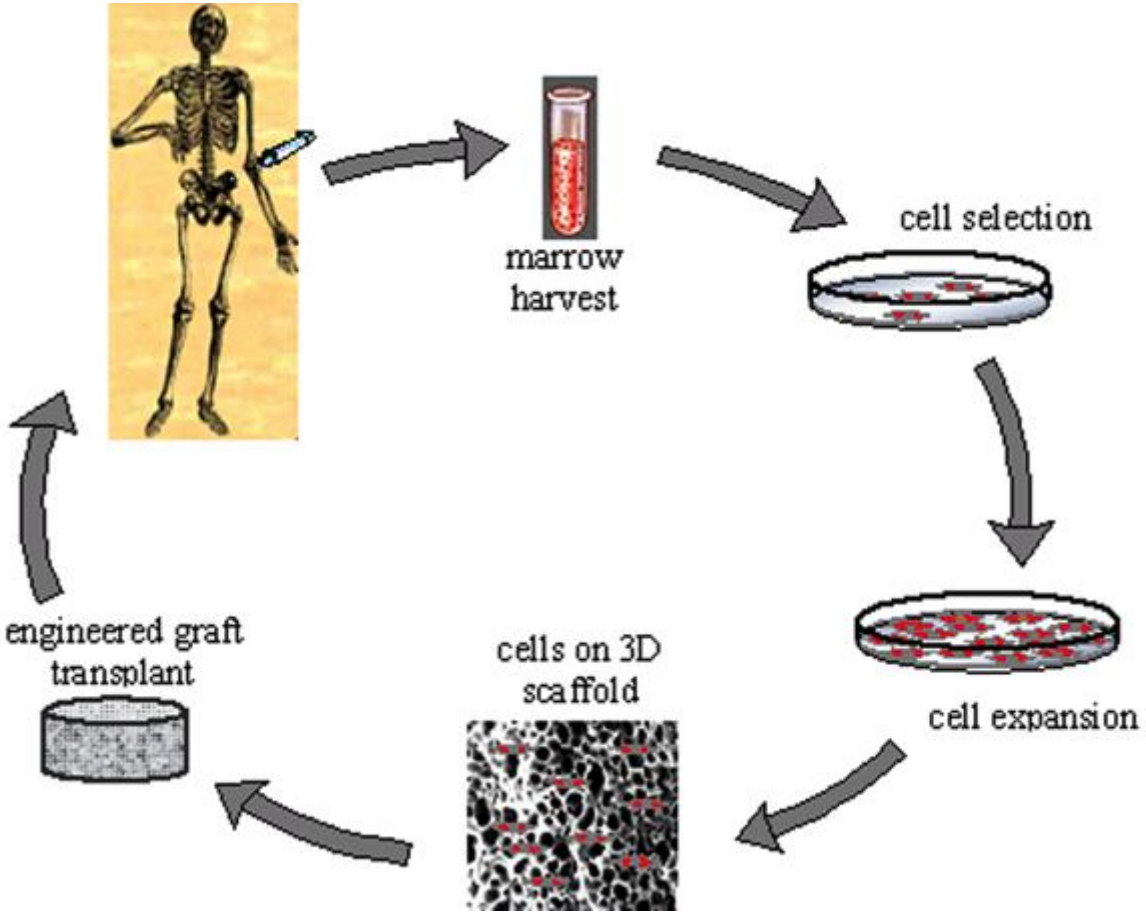
- Extracellular matrix analogues (EMA) are biomaterials that mimic in various ways the environment around bodily cells, with additional features that lead to desired developments, are obtained through a synthetic route are considered to be the new frontiers of biomimetic/bioactive biomaterials. They will be able to **control cell activities and tissue regeneration at nano-/micro-scale level**.

  Horizon 2020 Roadmap for Biomaterials

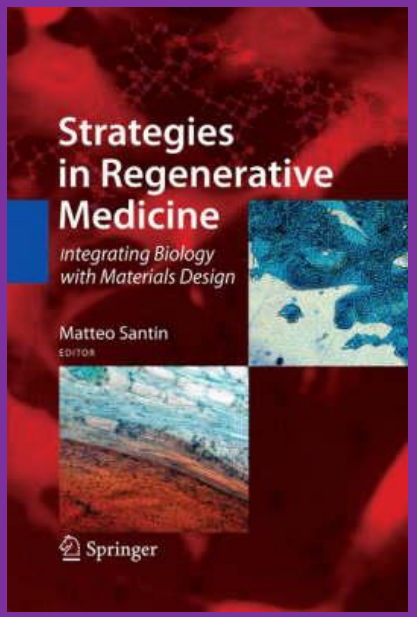
- Types of EMA
  - Nanostructured Biomimetic Materials
  - Bioactive Analogues of Growth Factors (Synthetic Pro-Morphogens)

University of Brighton

The Tissue Engineering Paradigm



From Scaglione & Quarto
In Strategies in Regenerative Medicine
M Santin ed, Springer 2009, Chapter 15



University of Brighton

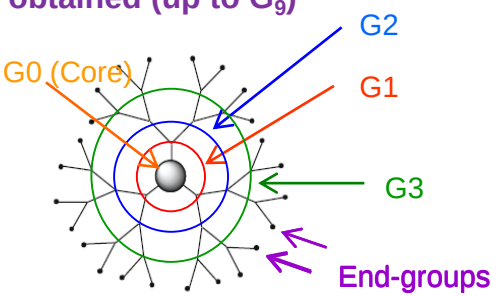
Biocompetent Dendrons

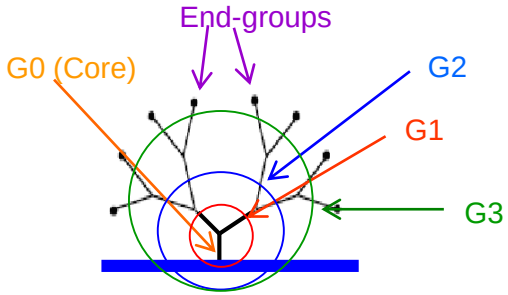
A Synthetic Biomaterial Platform to Mimic the Histological Lattice

University of Brighton

Dendrimeric Systems and Applications

- Dendrimers (Greek: δενδρον, tree) are hyperbranched polymers
- Dendritic structures with different branching generations (G_n) can be obtained (up to G_9)





- Dendrimer types
  - Poly(amido amine) [PAMAM]
  - Poly(ε-lysine)
  - Poly(ethylene glycol)
  - Polysaccharides-based
  - Nuceic acid-based

- Biomedical applications
  - Drug/gene carriers (Non viral carrier for *in vitro* cell transfection, Superfect™)
  - Contrast agents
  - Fibroblast spheroids (RGD-PAMAM)

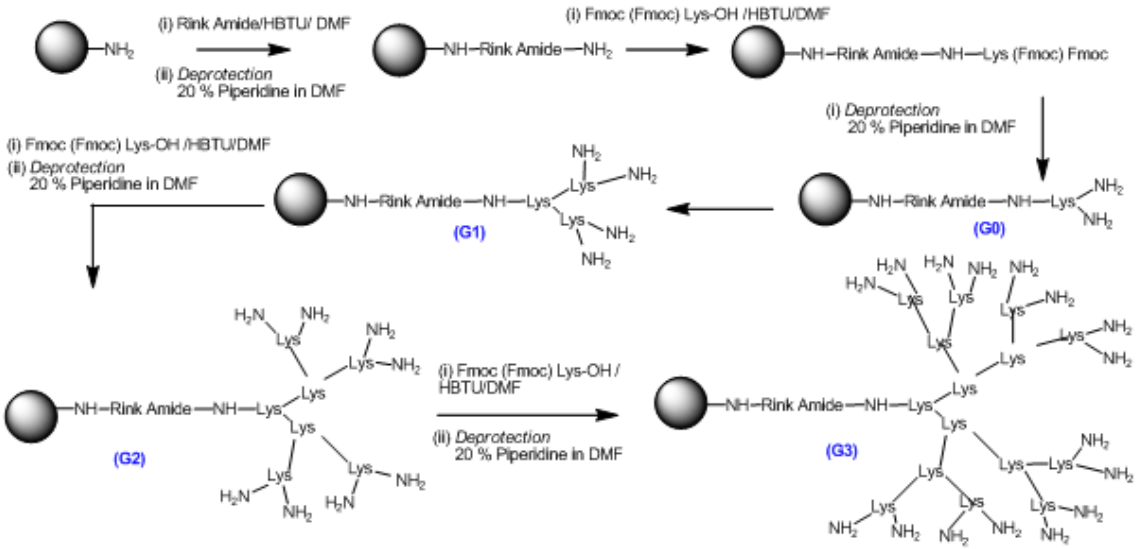
Al Jamal, K. T. et al, 2005, *Advanced Drug Delivery Reviews* 57(15), 2238-2270.

Duncan, R. & Izzo, L. 2005, *Advanced Drug Delivery Reviews*, 57(15), 2215-2237

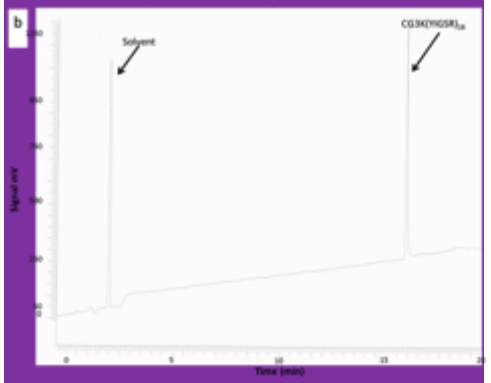
Tang M.X. *et al*. 1996, Bioconjugate Chemistry 7, 703-714

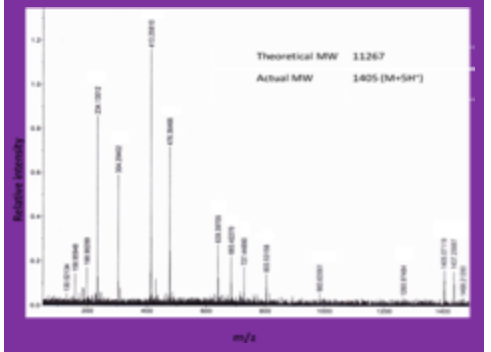
Materials and Methods:
Synthesis of Poly(ε-lysine) Dendrons

G3 Poly(ε-lysine) Synthesis



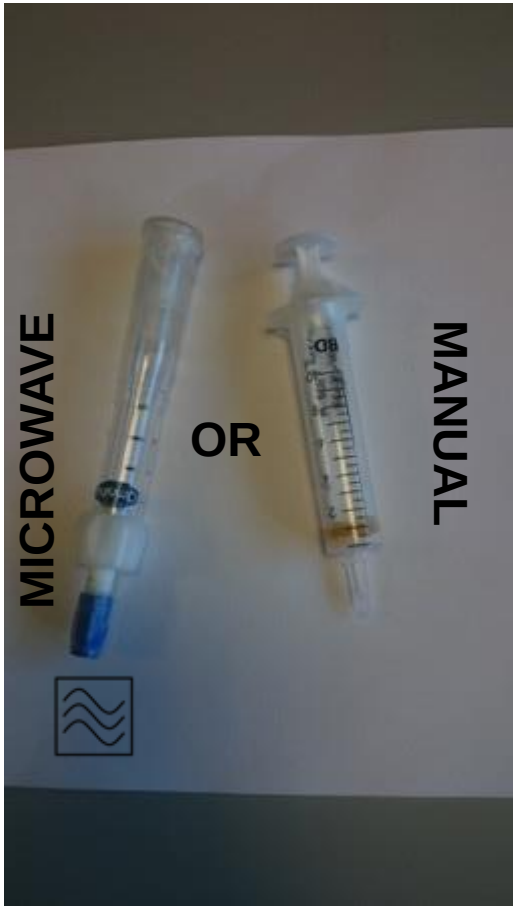
Microwave solid-phase synthesis
Scale up: ca 50 mg/batch
Purity > 95%

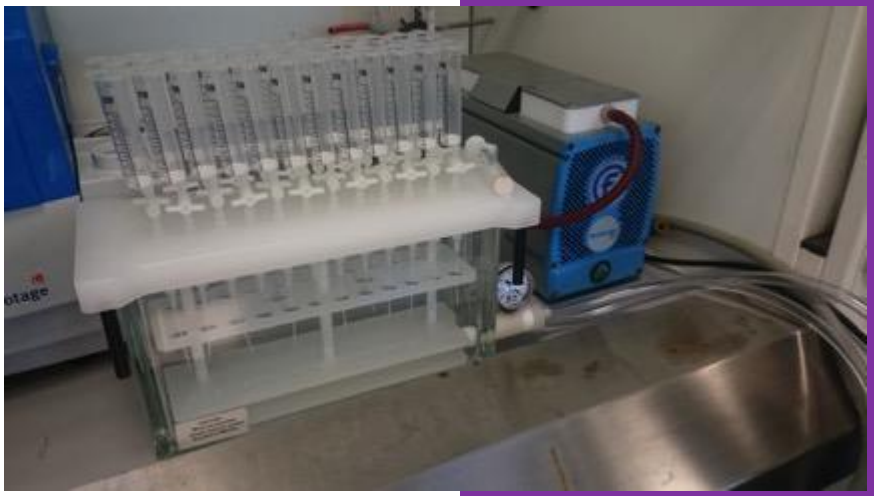




University of Brighton

Dendron Synthesis Scale up





24 X Scale Up

Microwave = ~7mins coupling

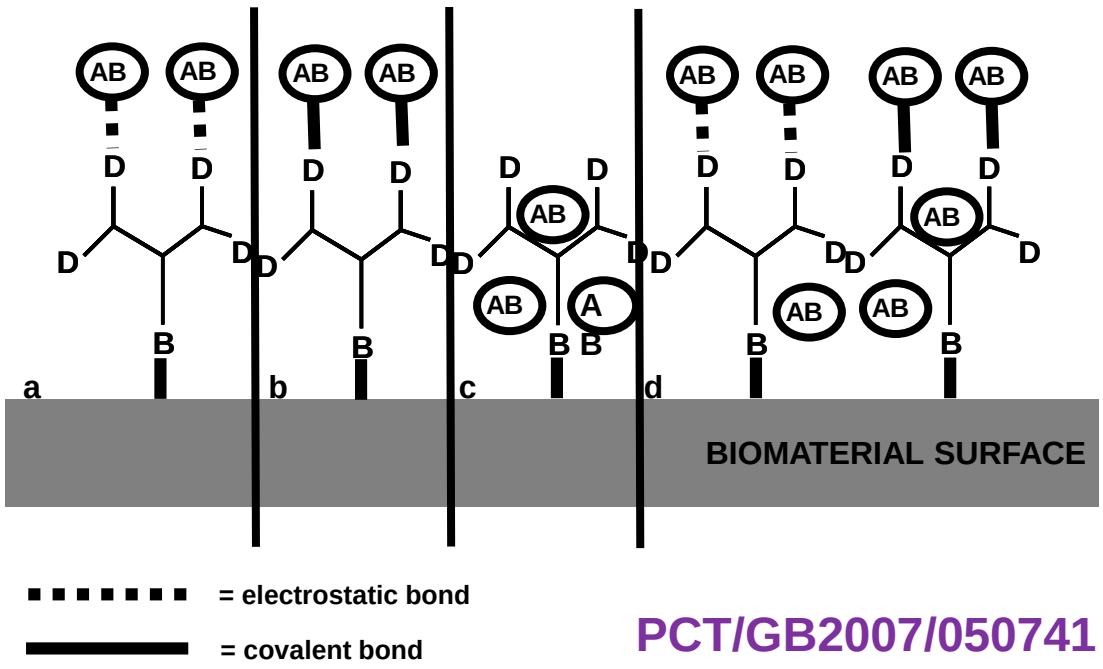
Manual = 30mins coupling

1 Syringe: 25% yield = ~250mg

24 Syringe = ~6000mg

University of Brighton

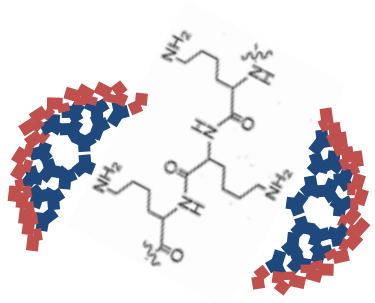
Biomimetic Dendrons as Surface Functionalisation Macromolecules



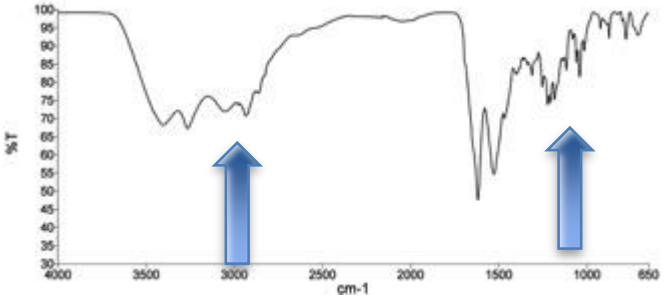
PCT/GB2007/050741

University of Brighton

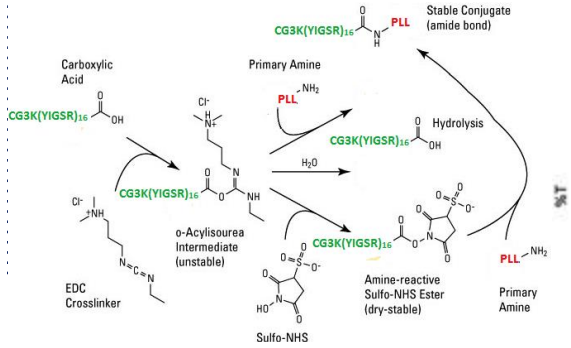
Dendron-modified poly(ε-lysine) substrates

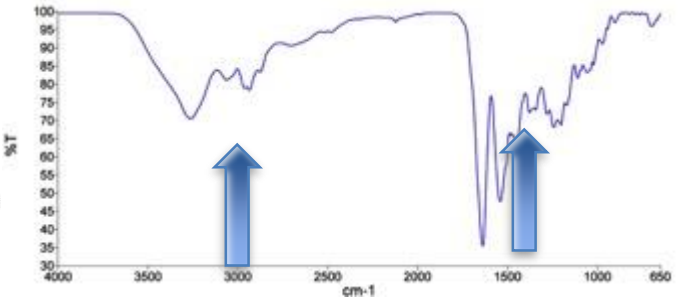


1% mol/-NH_2 mol



poly(K)





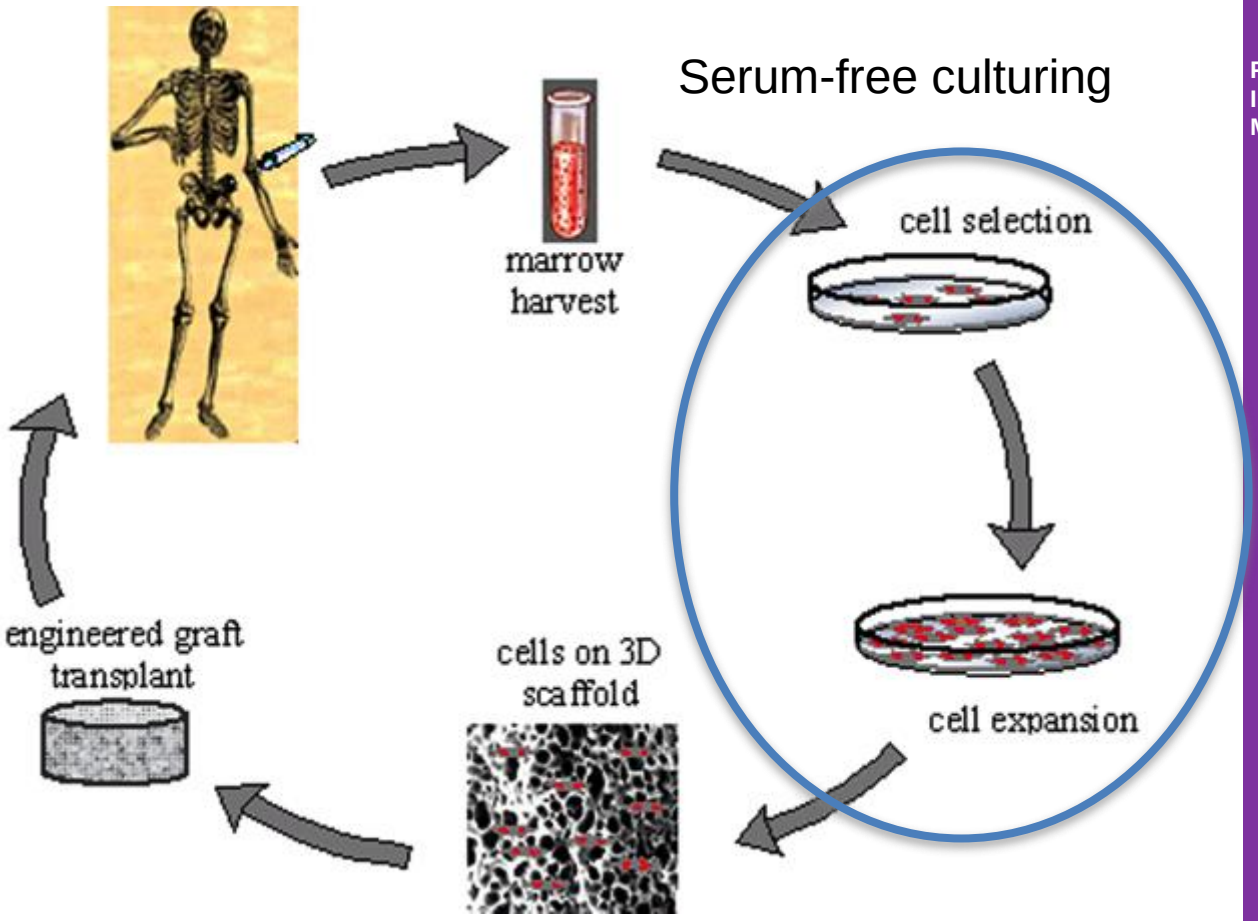
$RG_3K(YIGSR)_{16}$-poly(K)

University of Brighton

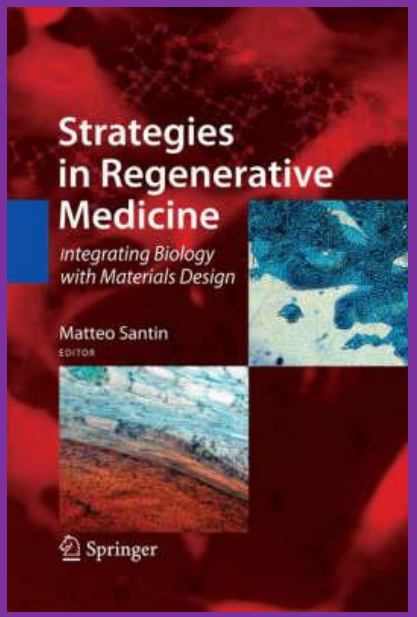
Extracellular Matrix Analogues for the Pre-clinical Handling of Cells

University of Brighton

The Tissue Engineering Paradigm

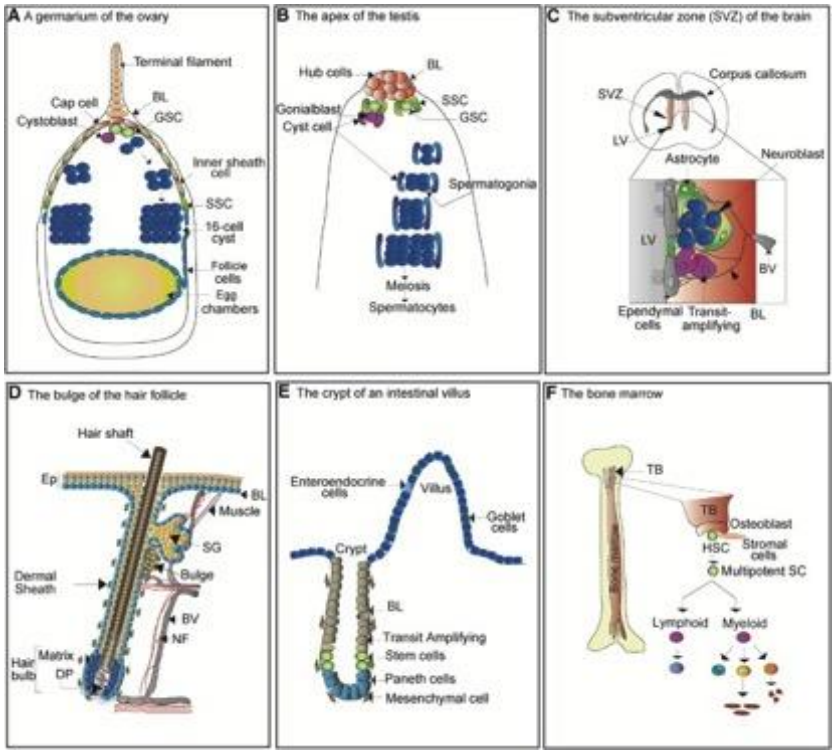


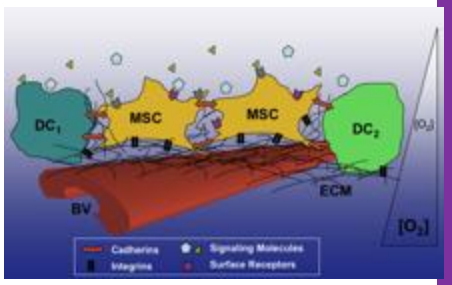
From Scaglione & Quarto
In Strategies in Regenerative Medicine
M Santin ed, Springer 2009, Chapter 15



University of Brighton

Types of Adult Stem Cell Niches

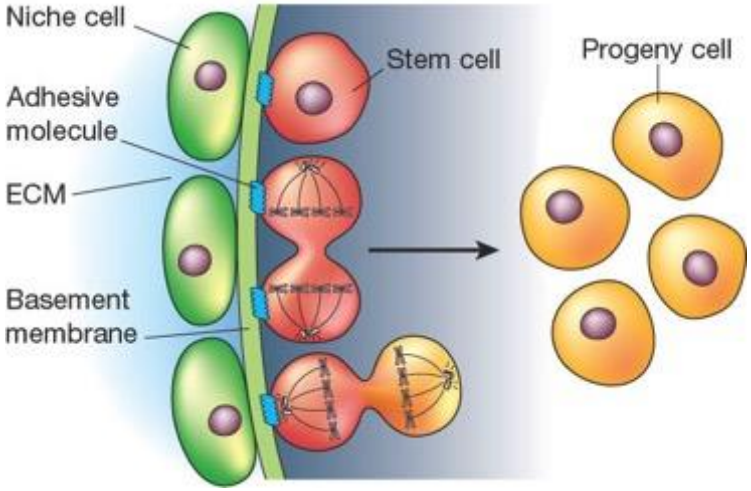


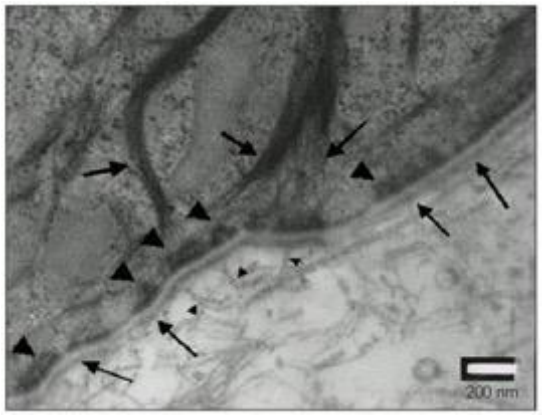


Sciencedirect.com

University of Brighton

The Basement Membrane

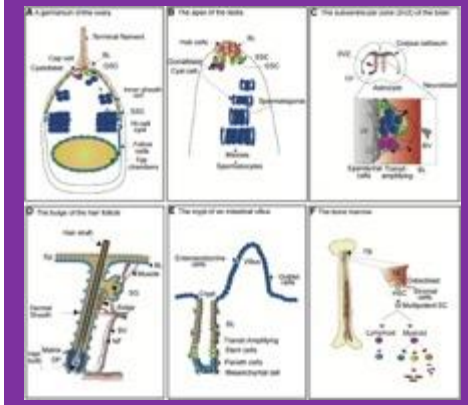




BMS ultrastructure

The major constituents of all BMs are:

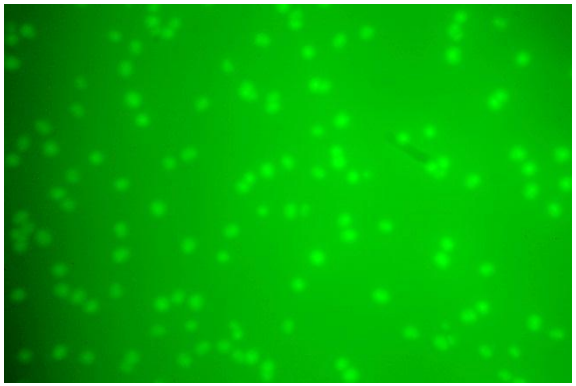
- **collagen IV**
- **laminins**
- **nidogen/entactin**
- **proteoglycans**



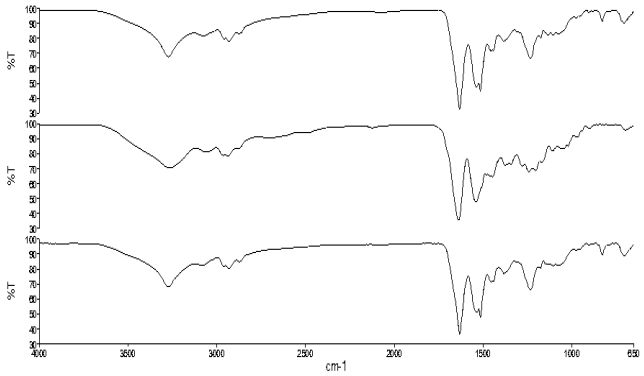
Poly(K) derivatisation method:
Activation of dendritic carboxyl groups by
10mM N-hydroxysulfossucinimide (sulfo-NHS)
4mM 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC)
Poly(K) (70,000-150,000) concentration: 0.1% (w/v)
Dendron concentration: 0.1% (w/v)
1 hour, room temperature.

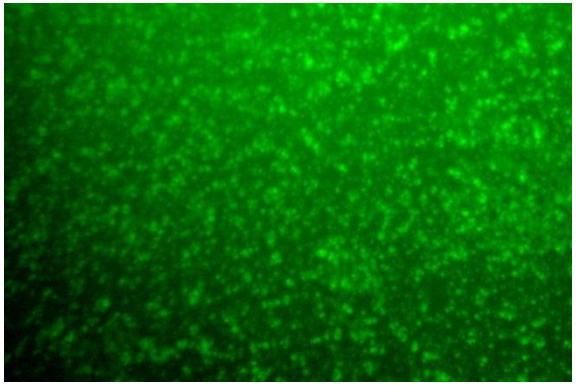
University of Brighton

Dendron-modified poly(ε-lysine) substrates

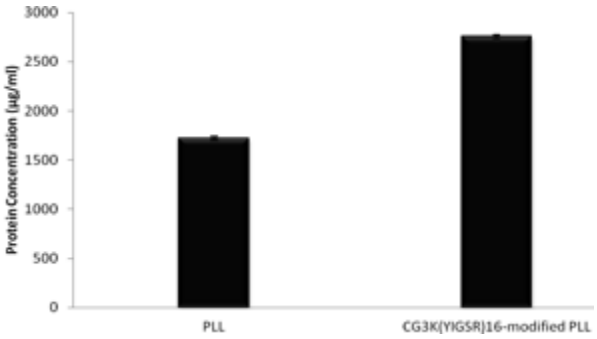


FITC-Labelled poly(K)



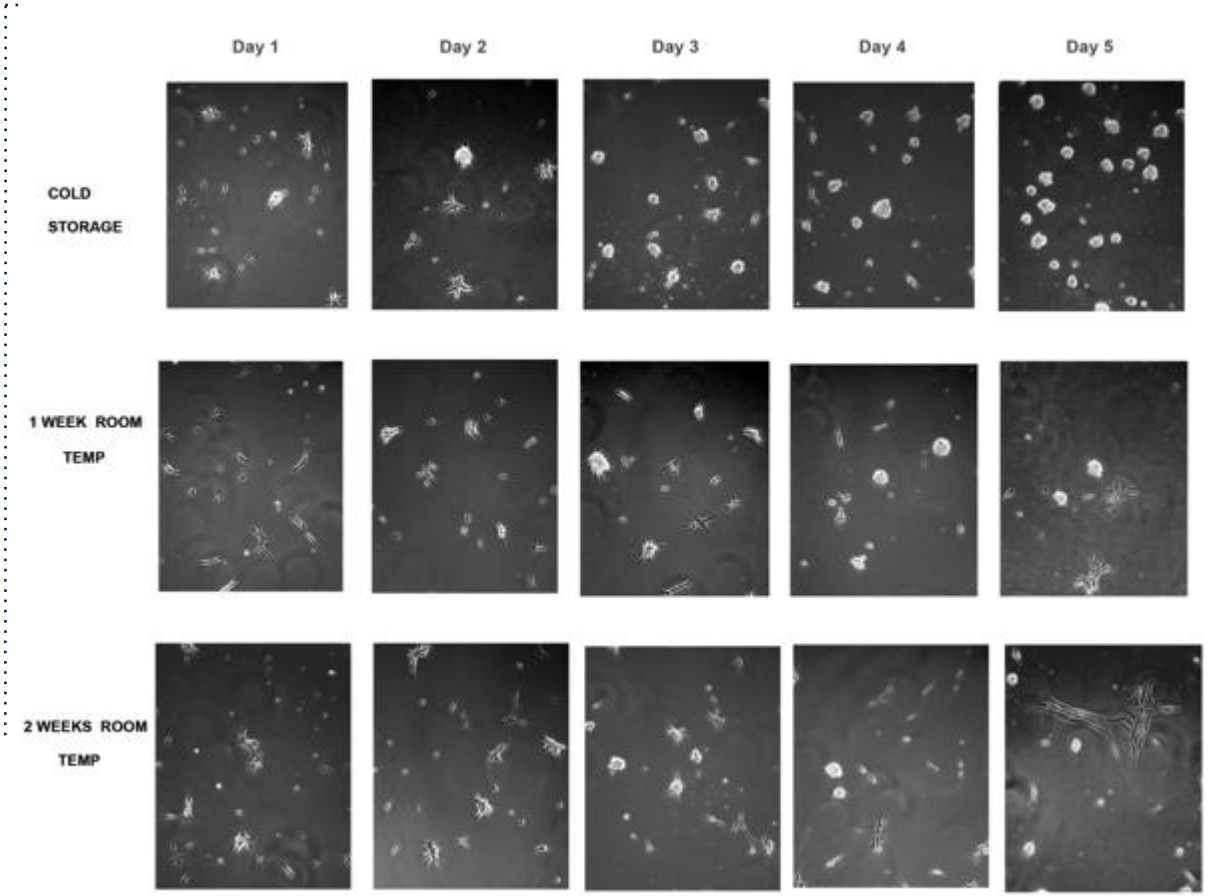


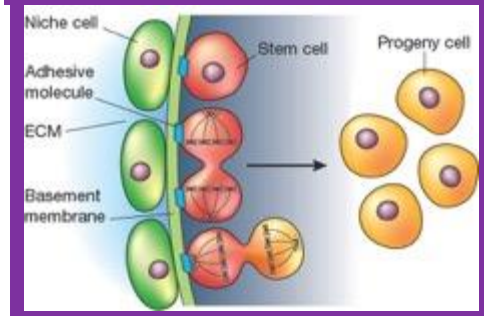
FITC-labelled $CG_3K(YIGSR)_{16}$



University of Brighton

Mesenchymal Stem Cell Adhesion on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate





University of Brighton

Mesenchymal Stem Cell Adhesion on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate

Tissue Culture Plastic

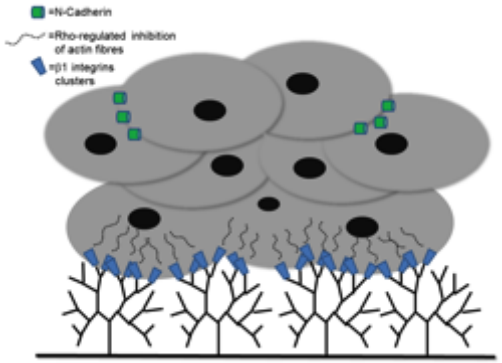
$CG_3K(YIGSR)_{16}$-poly(K)

0.5h

1h

3h

24h



University of Brighton

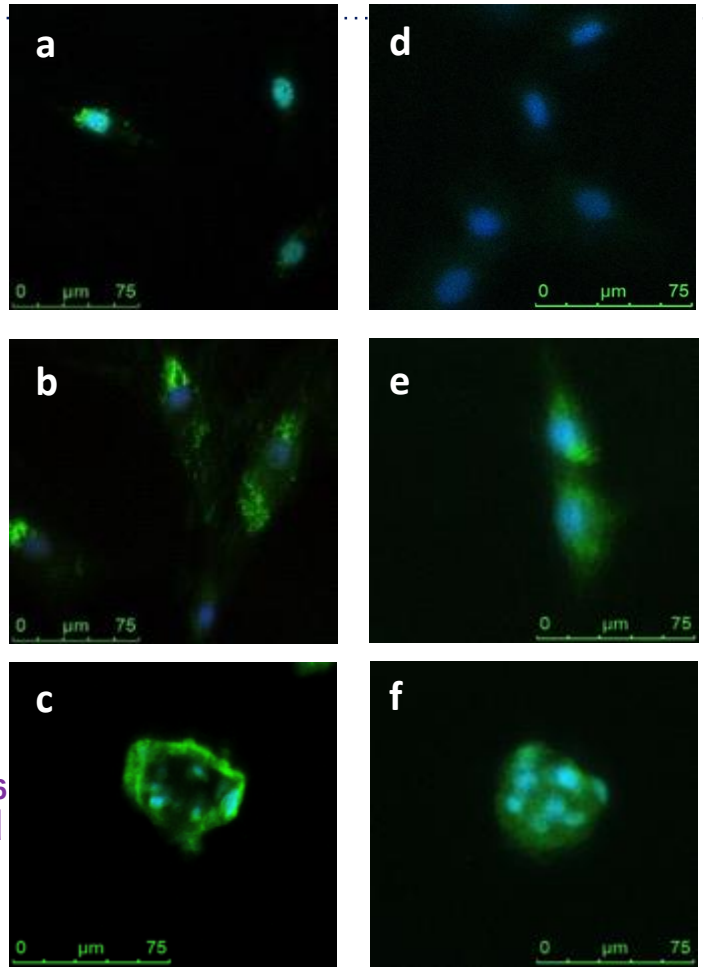
Integrin and Rho Expression and Localisation in Stem Cells Adhering on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate

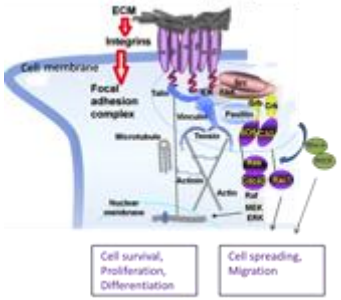
β-integrin | Rho-A

TCP

PolyK

$CG3K(YIGSR)_{16}$-functionalised PolyK

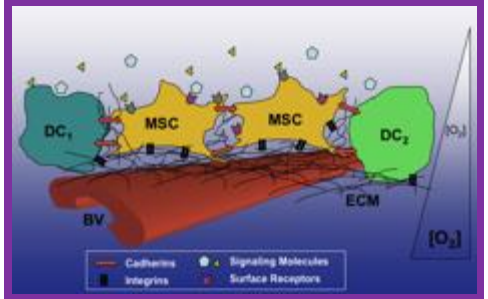




University of Brighton

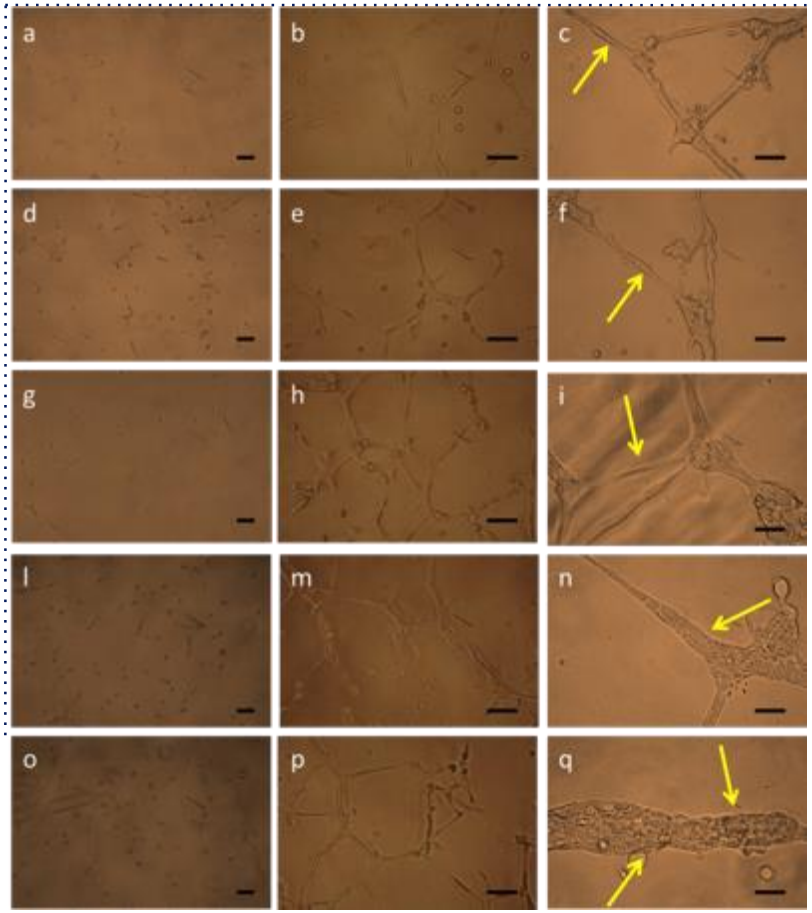
Expression and Localisation of Migration and Hyoxia Markers in Stem Cells Adhering on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate

	N-Cadherin	HIF-1α
TCP	a	d
PolyK	b	e
$CG3K(YIGSR)_{16}$-functionalised PolyK	c	f



University of Brighton

Stimulation of Endothelial Sprouting in 3D Matrigel by Supernatants of MSC Cultured on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate



2 days

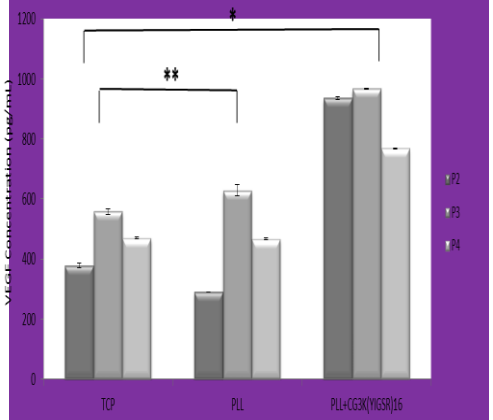
3 days

5 days

Tissue Culture Plastic

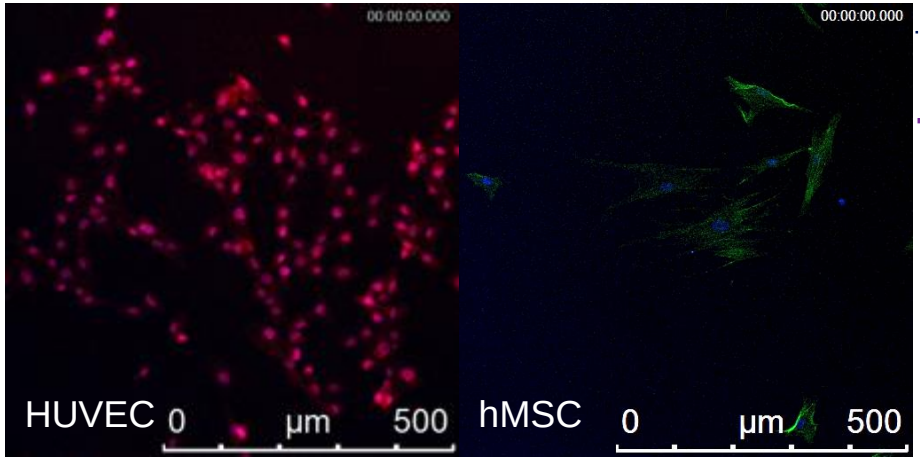
Poly(K)

$CG_3K(YIGSR)_{16}$-poly(K)



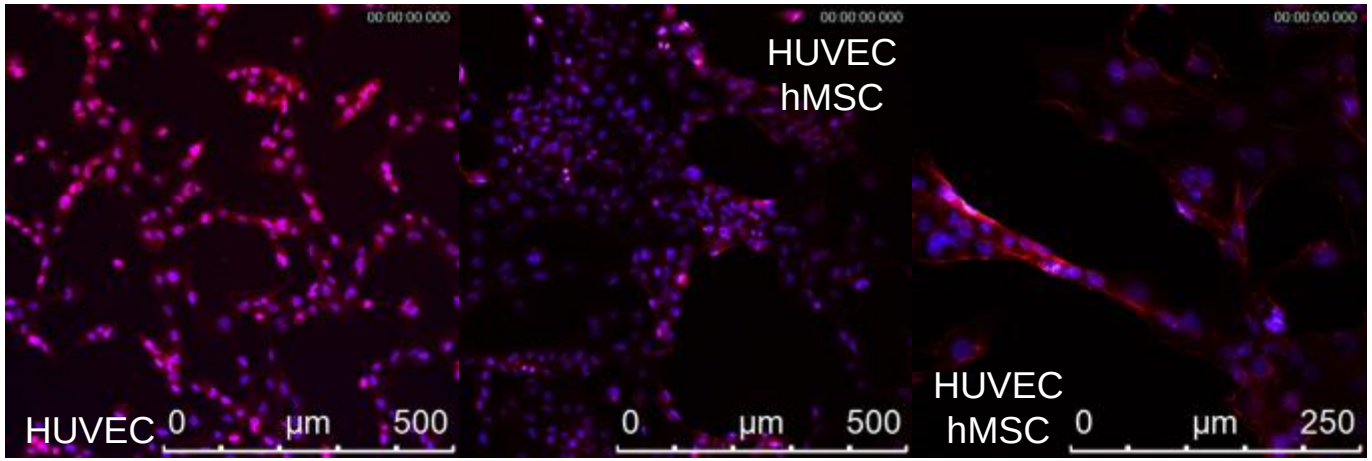
University of Brighton

Stimulation of Endothelial Sprouting on TCP by HUVEC/hMSC Co-Culture on $CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate



Tissue Culture Plate

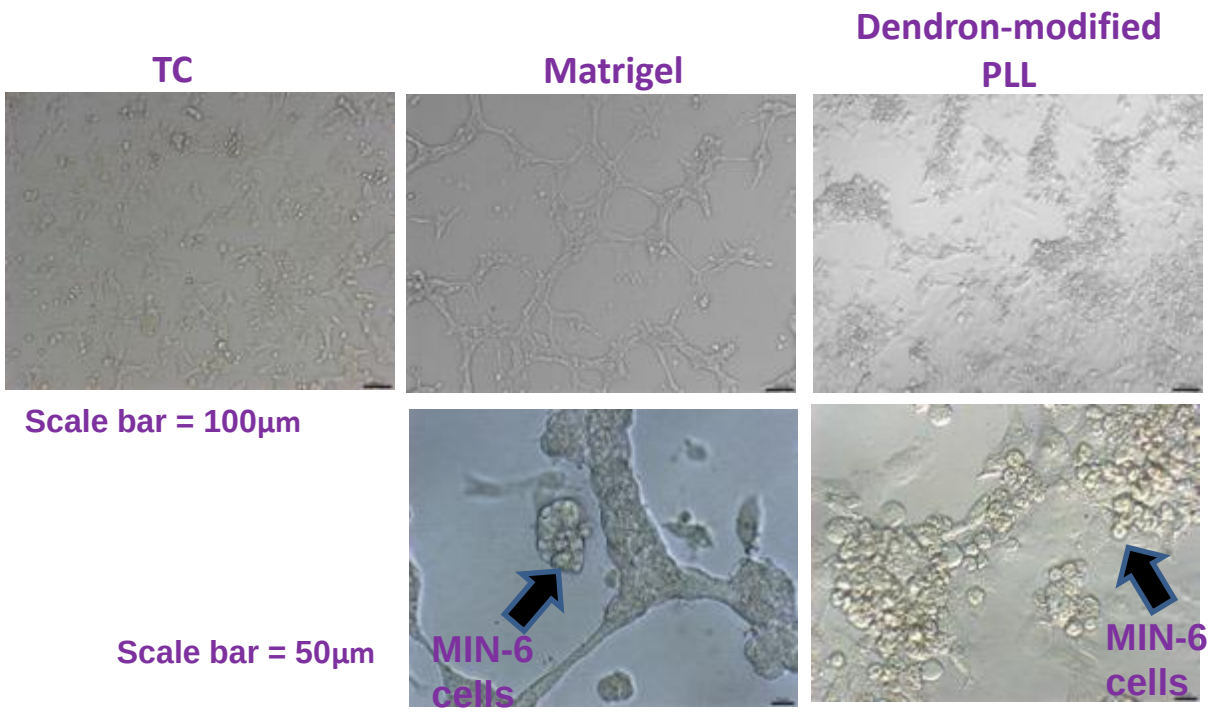
CD31 = HUVEC
CD44 = hMSC
DAPI = Nuclei



$CG_3K(YIGSR)_{16}$-tethered Poly(K) Substrate

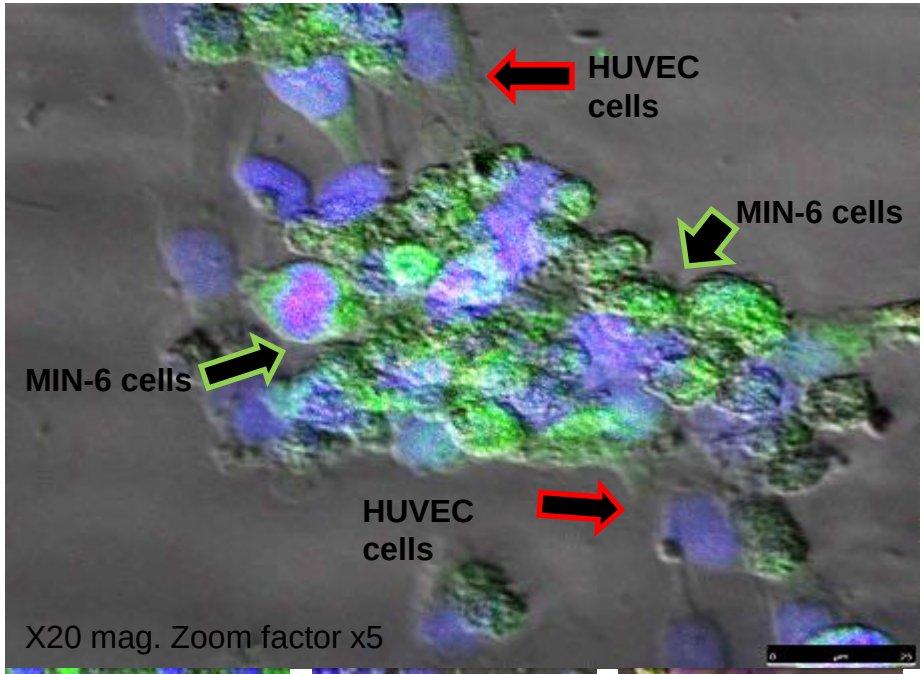
University of Brighton

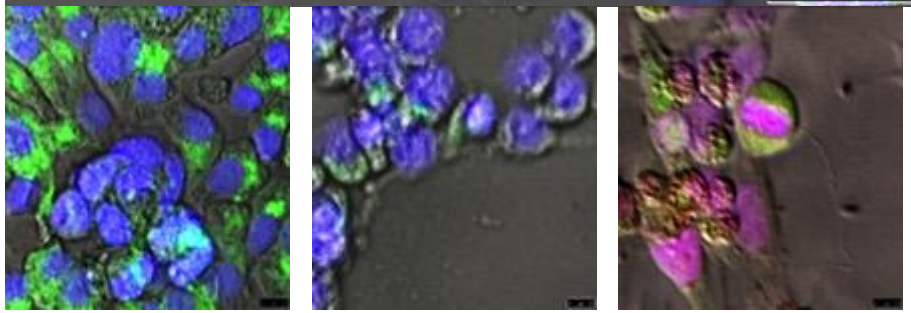
Beta Cells/Endothelial Cells Constructs



University of Brighton

Beta Cells/Endothelial Cells Constructs





DAPI = nucleus
Insulin
CD31
(endothelial marker)

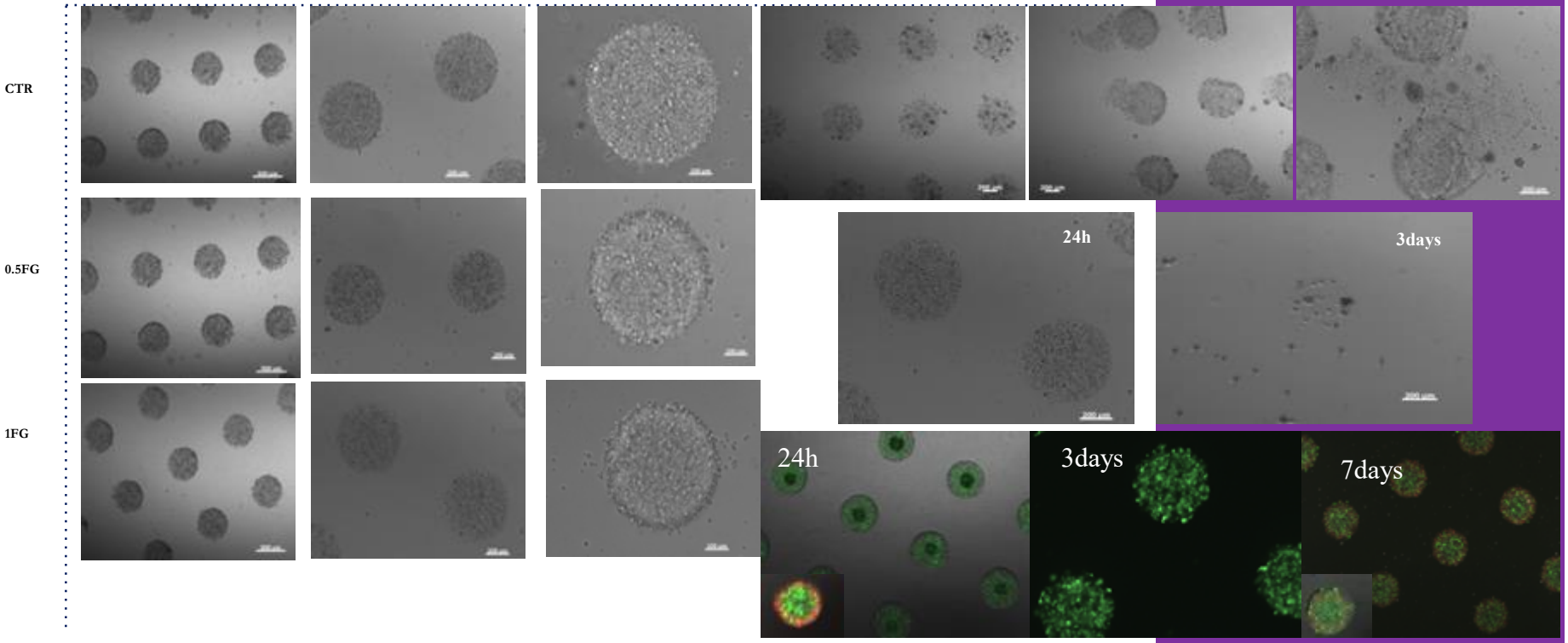
PLL-RG3K(YIGSR)16
20mins FBS free
TCP
FBS addition
40:60%
MIN-6: HUVEC medium
24h

*Scale bar = 25μm

*Scale bar = 7.5μm

University of Brighton

Printed Neuro-mimicking Constructs



Neural Cells Printed in Gel-MA and Gel-Ma functionalised with different concentrations of $CGen_3K(IKVAV)_{16}$

University of Brighton

Phenodrives

Phenodrive	Stem Cells	Endothelial Cells	Neural Cells	Epithelial cells	Osteoblasts	Chondrocytes	Haepatocytes	Cancer Cells
Integrin YIGSR	✔	✔		✔				
Integrin IKVAV			✔					
Integrin RGD				✔	✔			
Universal Carboxybetaine	✔					✔	✔	✔
PS					✔			
Hypoxia						✔	✔	✔

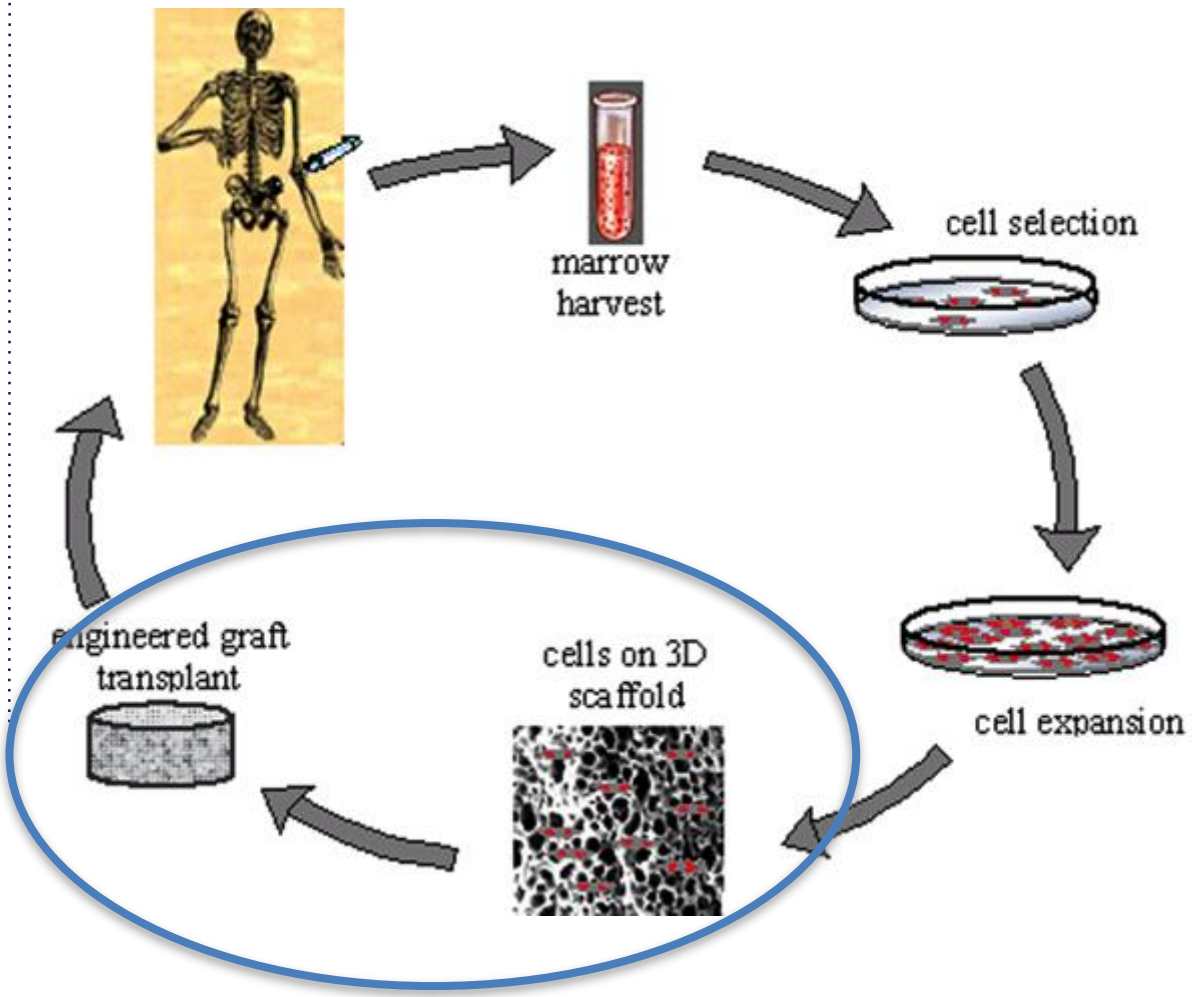
www.tissueclick.com
info@tissueclick.com

University of Brighton

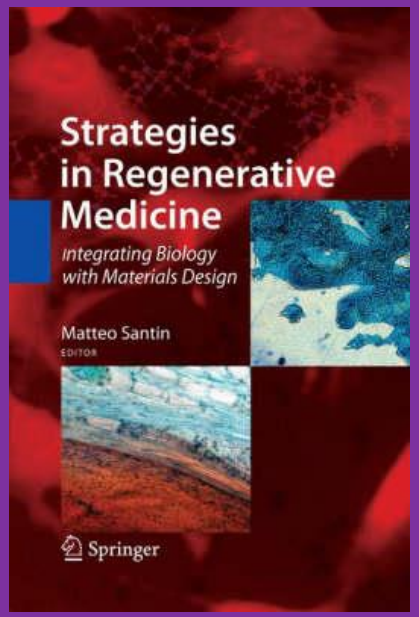
Functionalisation of 3D Scaffolds for Osteochondral Defect Regeneration

University of Brighton

The Tissue Engineering Paradigm

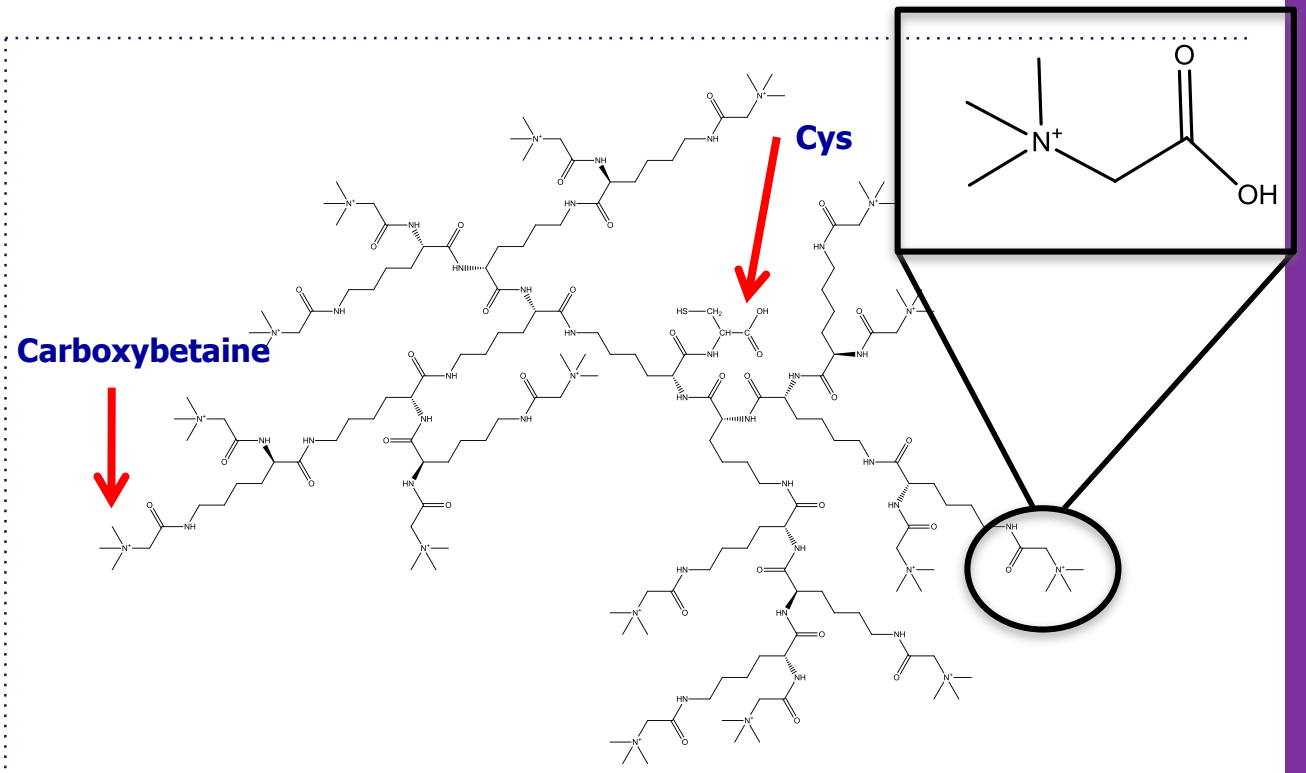


From Scaglione & Quarto
In Strategies in Regenerative Medicine
M Santin ed, Springer 2009, Chapter 15

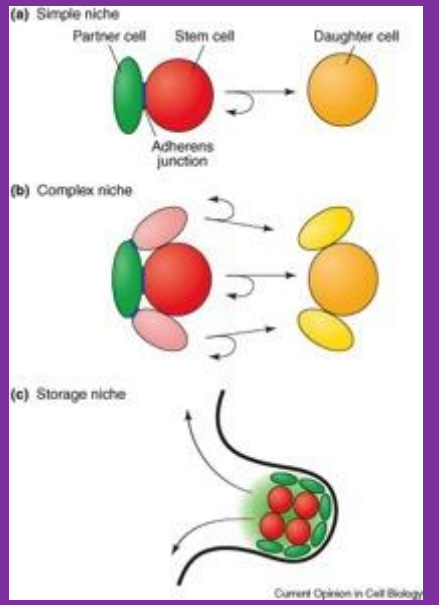


University of Brighton

Docking Site for Migrating/Loaded Stem and Differentiated Cells: The C $G_3K(Carboxybetaine)_{16}$ Unit

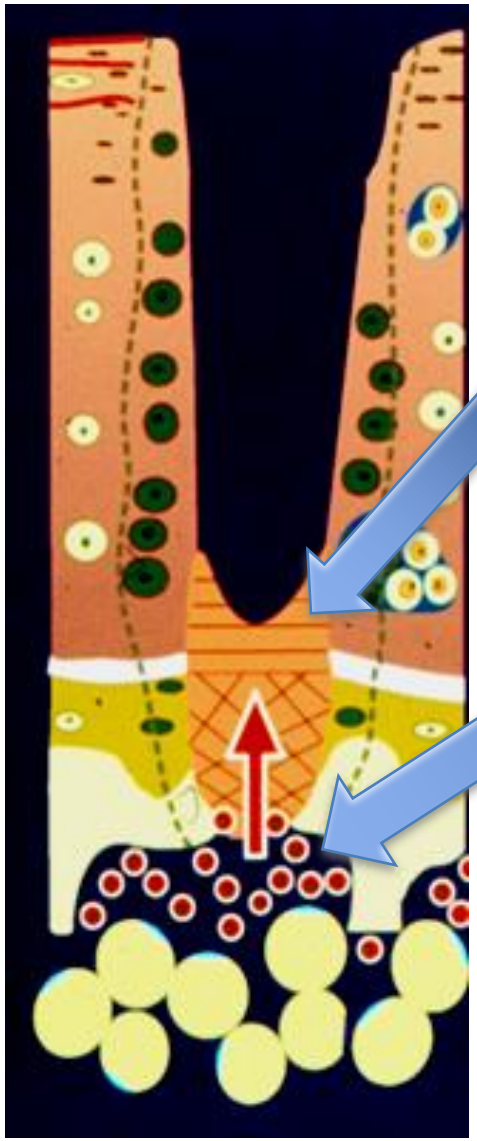


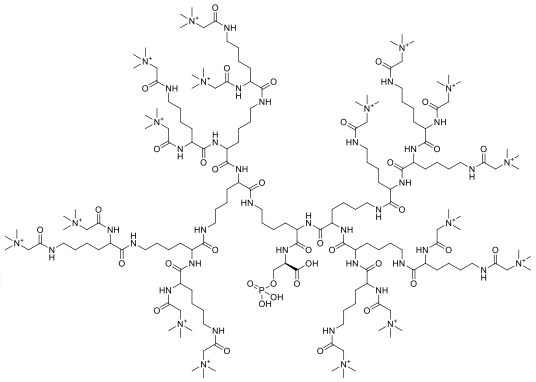
Chemical Formula: $C_{173}H_{347}N_{47}O_{33}S^{16+}$
Exact Mass: 3643.66
m/z: 3644.67 (100.0%), 3645.67 (97.4%), 3646.67 (81.0%), 3643.66 (53.4%), 3647.68 (25.8%), 3647.67 (21.1%), 3645.66 (19.8%), 3648.68 (12.4%), 3644.66 (9.7%), 3648.67 (6.1%), 3646.66 (6.0%), 3649.67 (1.9%), 3647.66 (1.7%)
Elemental Analysis: C, 56.99; H, 9.59; N, 18.06; O, 14.48; S, 0.88



University of Brighton

Docking Site for Migrating/Loaded Stem and Differentiated Cells

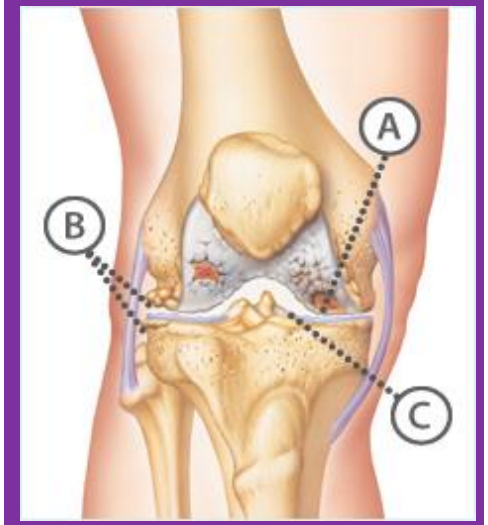




PS G3K $(CB1)_{16}$
FW = 3709

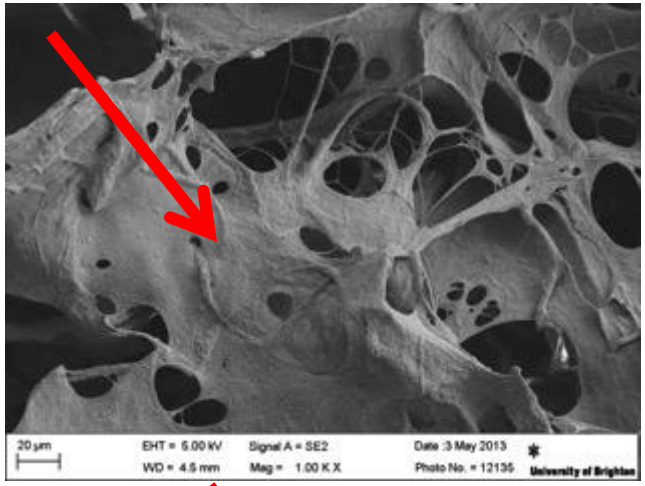
MaioRegen
Finceramica, Italy

Schematic Representation of an Osteochondral Defect and Tissue-competent Semi-dendrimers (Dendrons)

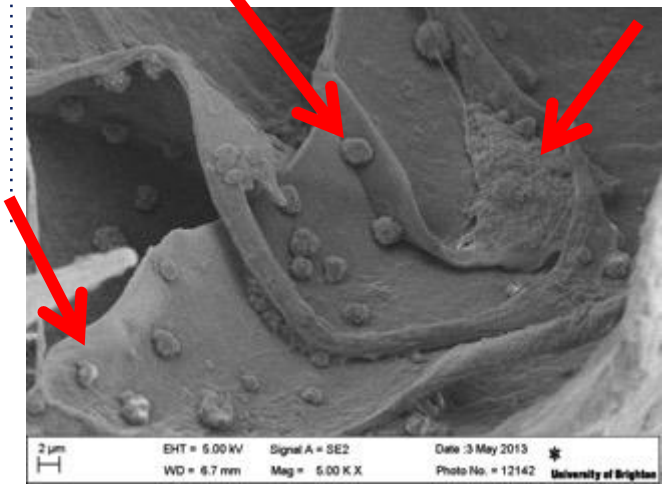


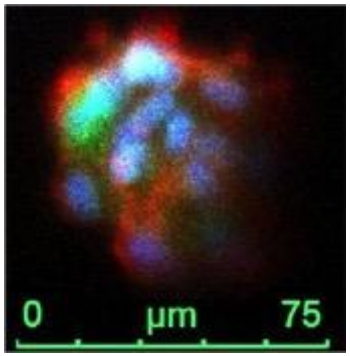
University of Brighton

Mesenchymal Stem Cell Organisation in Dendron-functionalised Scaffolds



MaioRegen
Finceramica, Italy





C G_3K $(Carboxybetaine)_{16}$-
MaioRegen

Surgery by
Dr E. Kon , Dr G.Filardo
IOR, Bologna,
Italy

University of Brighton

RAPID MAGNETISATION OF MESENCHYMAL STEM CELLS

Nunzia Di Maggio[1*], Elisa Martella[2,3*], Steve Meikle[4], Marta Columbaro[5], Enrico Lucarelli[2], Matteo Santin[4‡] and Andrea Banfi[1‡]

Rapid and efficient magnetization of mesenchymal stem cells by dendrimer-functionalized magnetic nanoparticles, Nanomedicine 2016, DOI 10.2217/nnm-2016-0085

[1]Cell and Gene Therapy, Department of Biomedicine, Basel University and Department of Surgery, Basel University Hospital, Basel, Switzerland, [2]Osteoarticular Regeneration Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy
[3]Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Italy
[4]BrightSTAR, Brighton Centre for Regenerative Medicine, University of Brighton, UK
[5]Musculoskeletal Cell Biology Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy

University of Brighton

Stem Cell Magnetisation

- Intraoperative production of tissue-engineered graft
  - isolation of multipotent progenitors from a patient,
  - their seeding on an appropriate scaffolding material
  - their re-implantation in a single-step procedure directly in the operating room

Mehrkens A, Saxer F, Guven S, *et al. Eur. Cell. Mater.* 24, 308–319 (2012).

- Magnetic scaffolds have been recently developed and cell magnetization can be exploited to rapidly and consistently drive cells into the scaffold prior to transplantation.

Yun H-M, Ahn S-J, Park K-R, *et al*. *Biomaterials*. 85, 88–98 (2016).

University of Brighton

Cell Magnetisation by Superparamagnetic nanoparticles

- Coupling of cells to superparamagnetic nano-particles (MNP) can confer a magnetic drive to the cells
- Cell labelling with MNP is a slow and low-yield process (12 to 24 h) often not able to guarantee levels of magnetization sufficient for cell manipulation
- MNP (from 5 to 150 nm in diameter) can be coated with different polymeric materials (e.g. dextran, polylysine, chitosan or silica) to enhance their biocompatibility and promote their endocytosis

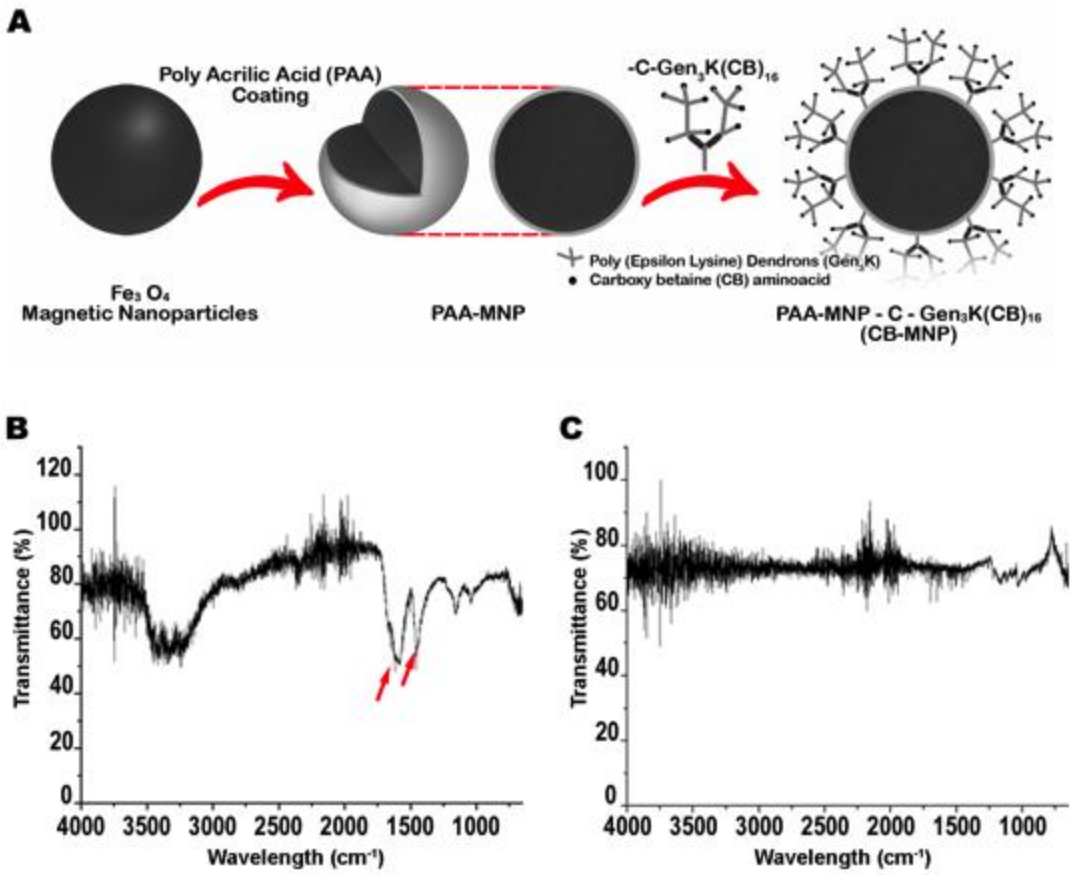
University of Brighton

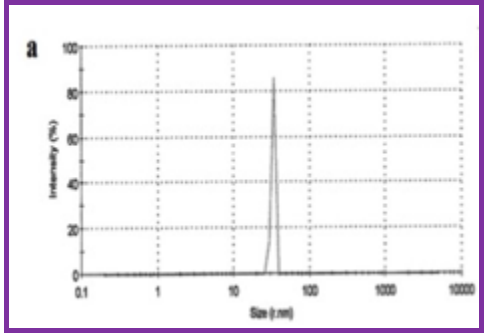
Materials and Methods: Optimised $CG3KCB_{16}$MNP@PAA manufacturing

- PAA@MNP were functionalised with Cys via formation of an amide bond using carbodiimide/N-hydroxysuccinimide chemistry (4:1) ratio in 0.1 MES buffer for 1 hour in a shaking incubator (100 rpm, room temperature)
- Cys was added for 1 hour in a shaking incubator (100 rpm, room temperature). Supernatant was removed and beads washed with ethanol and air dried.
- $CG3KCB_{16}$ dendron was attached to PAA@MNP -Cys-SH via formation of –S-S- by 3 % H_2O_2 treatment for 2 hours. Supernatant removed and samples air dried
- Physico-chemical characterisation was performed

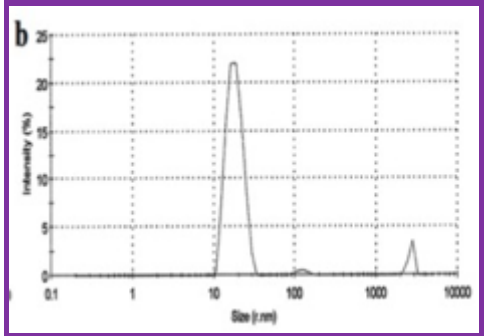
University of Brighton

PAA@MNP Functionalisation by carboxybetaine-tethered poly(ε-lysine) dendrons





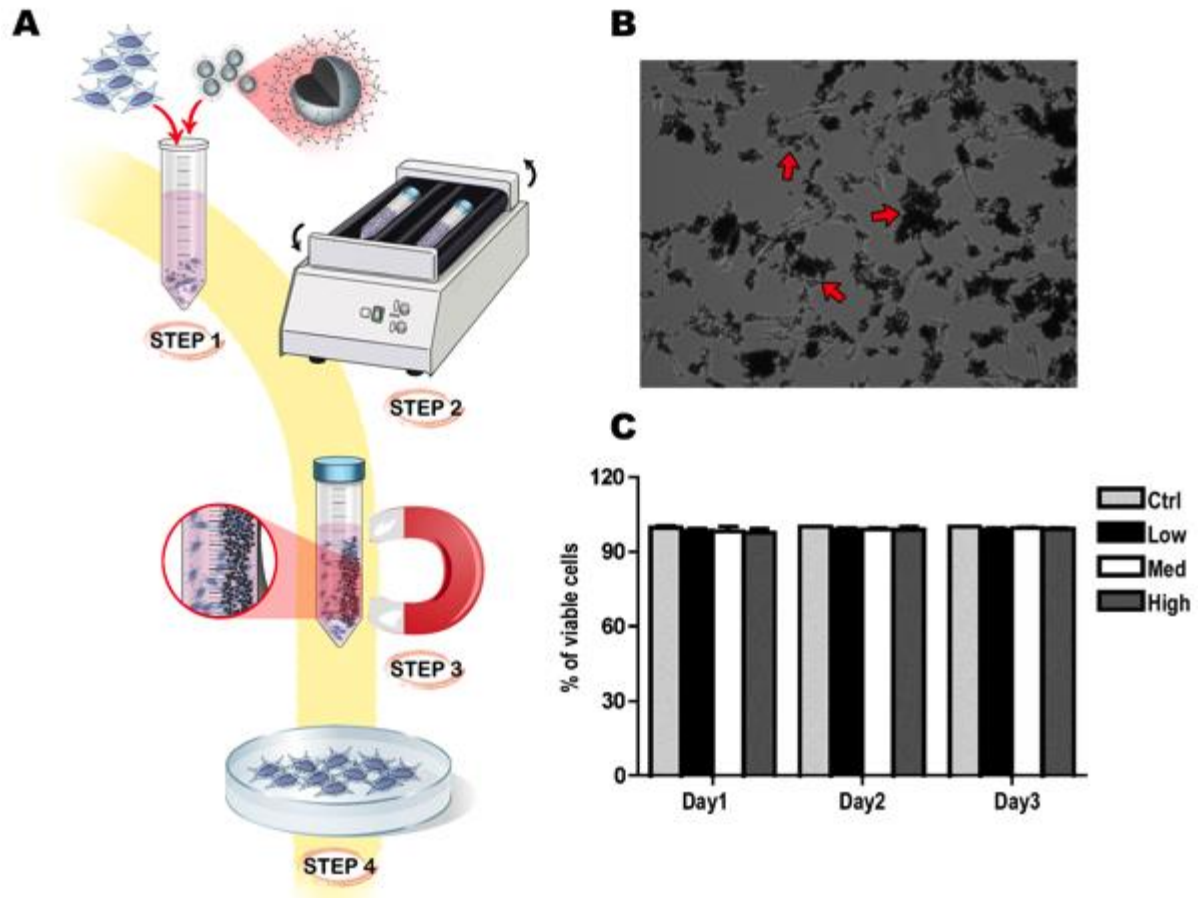
Size= 33.37 nm
Zeta Pot = -25.6 mV



Size= 18.46 nm
Zeta Pot = -45.6 mV

University of Brighton

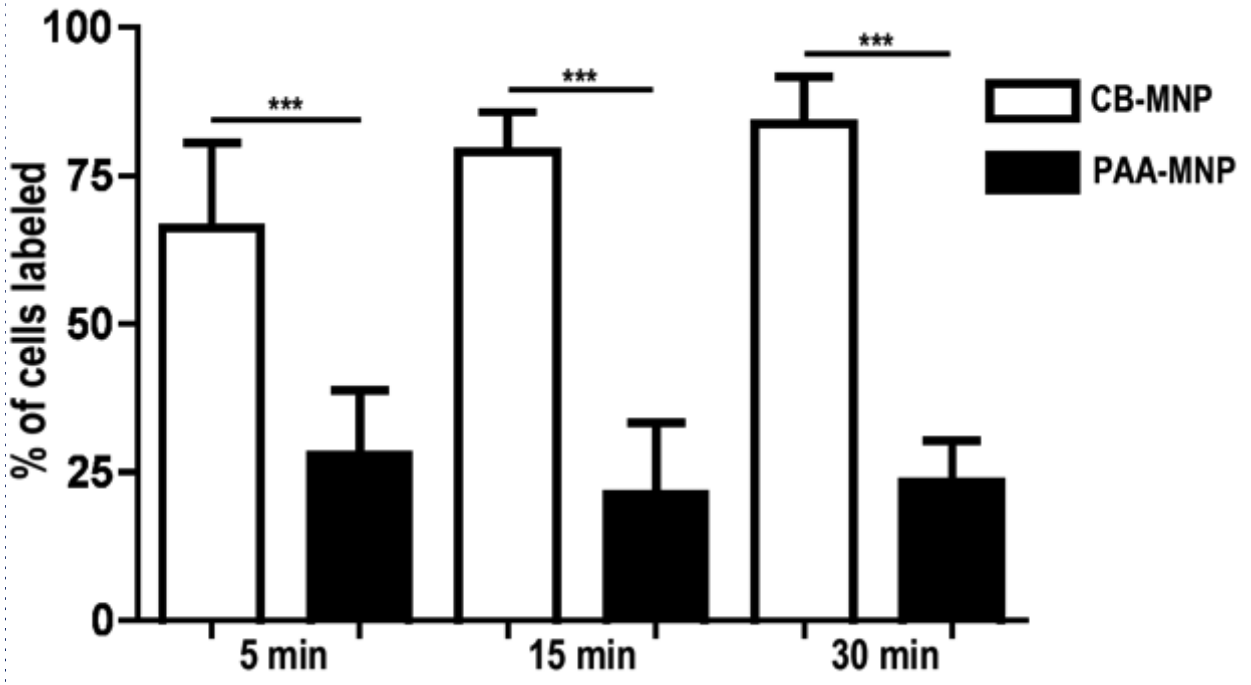
Mesenchymal Stem Cell Labelling with MNP



low = 7.2 µg/10^4 cells
medium = 14.4 µg/10^4 cells
high = 28.8 µg/10^4 cells

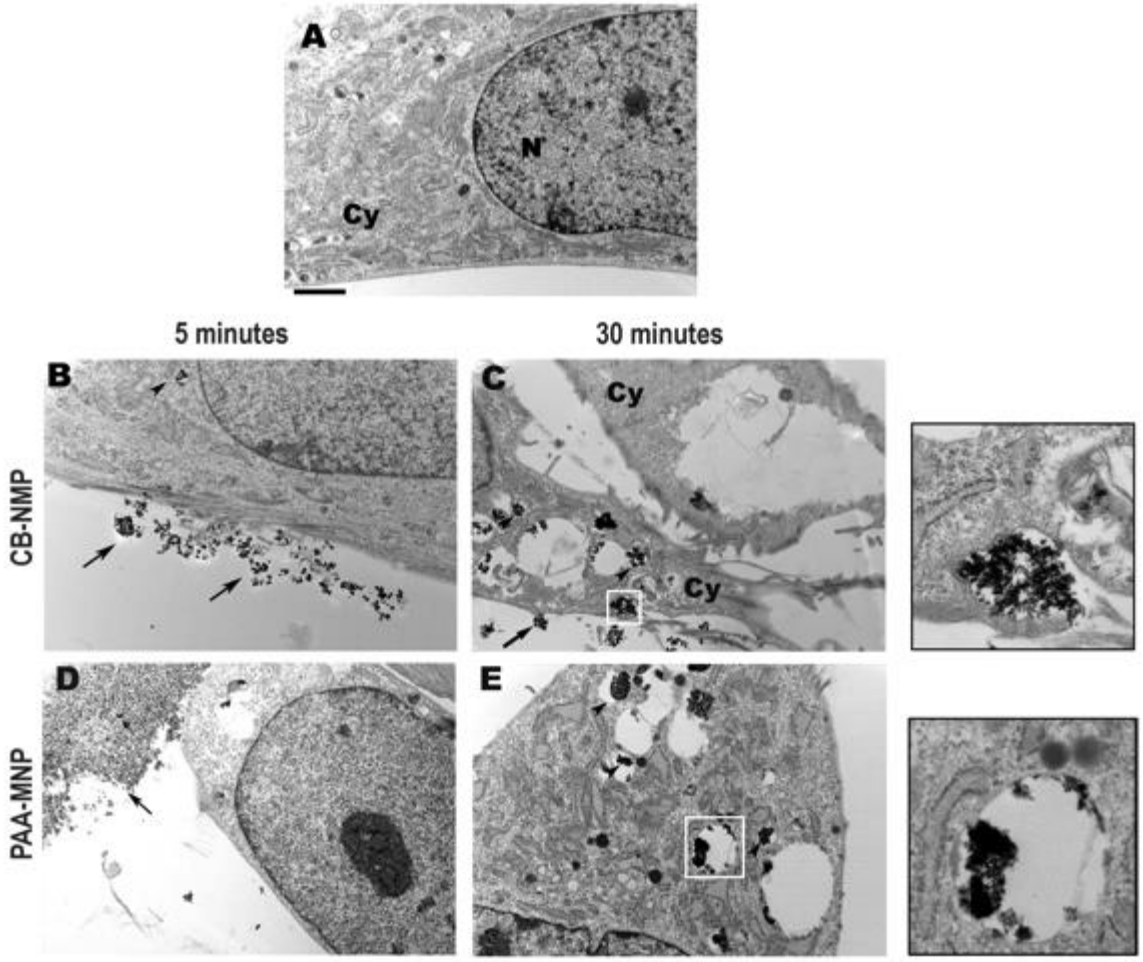
University of Brighton

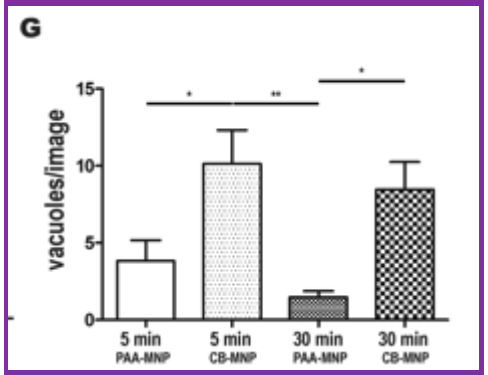
Mesenchymal Stem Cell Labelling with MNP



University of Brighton

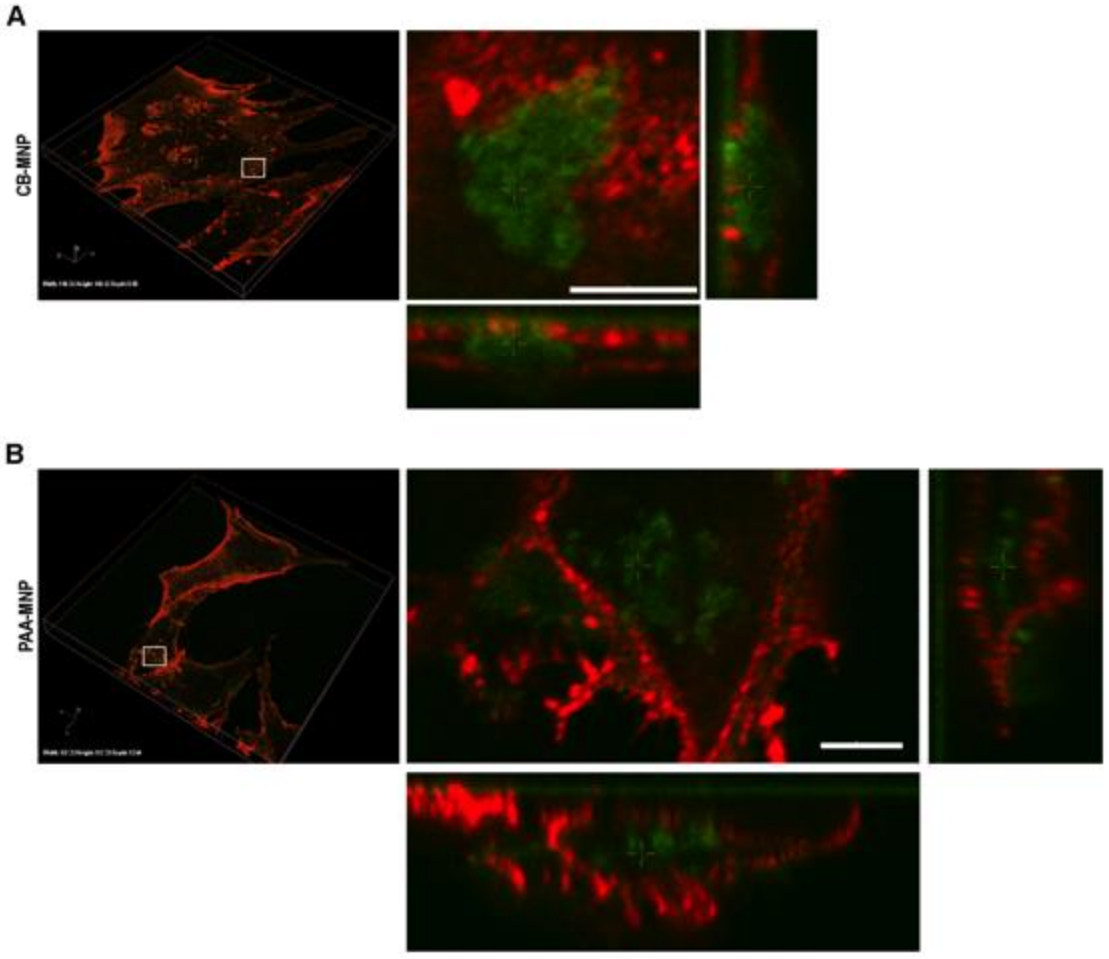
Mesenchymal Stem Cell Labelling with MNP: TEM Analysis

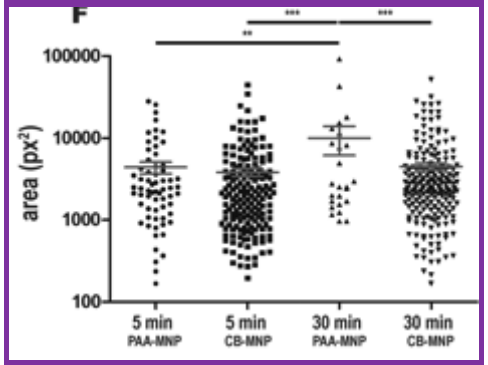




University of Brighton

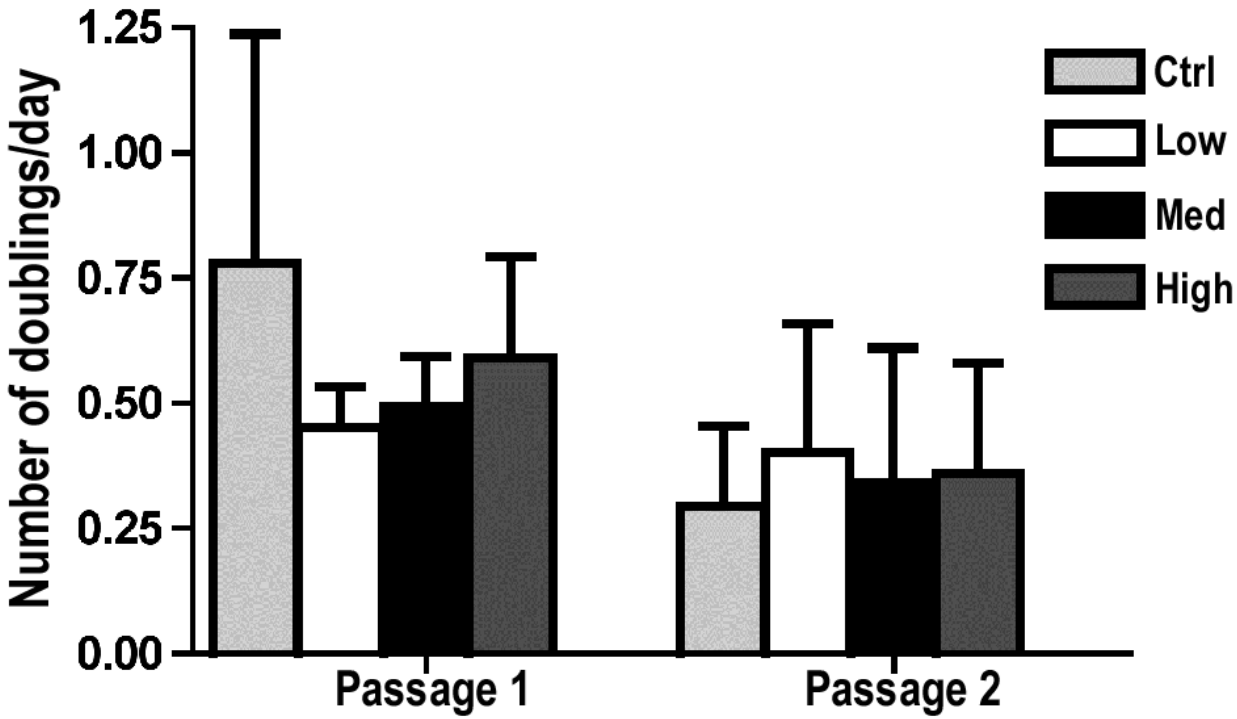
Mesenchymal Stem Cell Labelling with MNP: Confocal Analysis





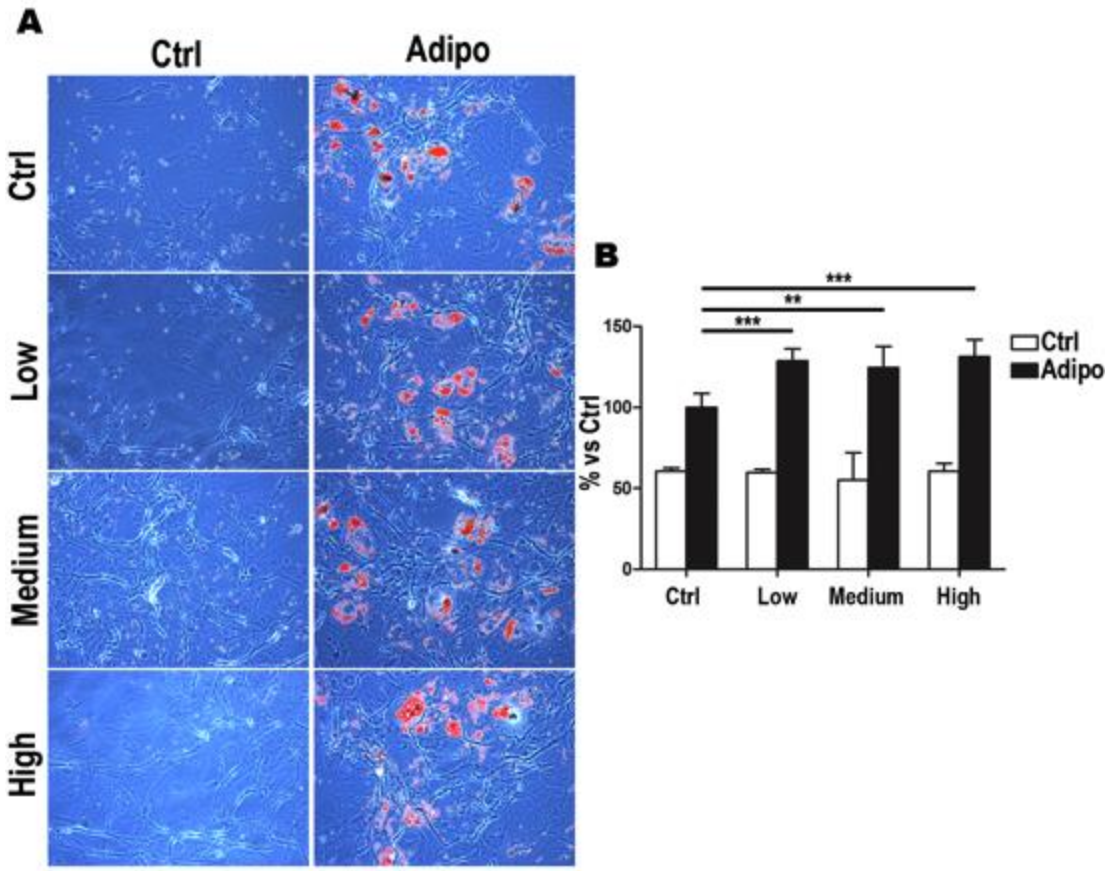
University of Brighton

Mesenchymal Stem Cell Labelling with MNP: Population Doubling



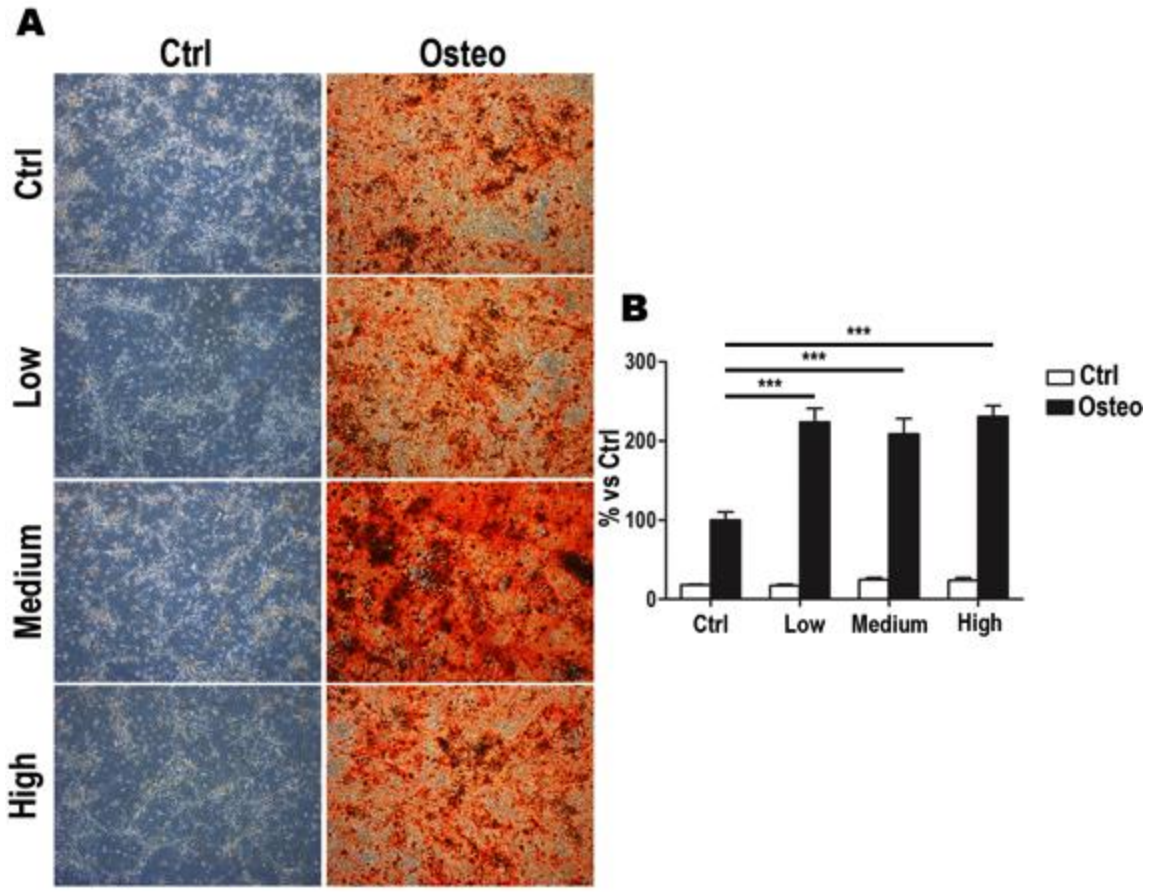
University of Brighton

Mesenchymal Stem Cell Labelling with MNP: Adipogenic Differentiation



University of Brighton

Mesenchymal Stem Cell Labelling with MNP: Osteogenic Differentiation



University of Brighton

Conclusions

- Synthetic Extracellular Matrix Analogues can be achieved by macromolecular design, scaled-up batches and **GMP conditions**
- EMA can be exploited as
  - Specialised substrates for cell pre-clinical handling of cells
  - more complex **clinically-reflective in vitro models** including different types of cells and integrated testing the safety/efficacy of drugs and other products in a regenerative niche
  - Functionalisation of 3D scaffolds
  - Bioink for additive manufacturing adding biofunctionality to a variety of more established polymers

University of Brighton

Acknowledgements

HEALTH.2013.1.3-2-602235

NMP-2009-2.3-1-246373

NEXT
Nano Engineering for Cross Tolerance
New approach for bioengineered chimeric pancreatic islet


G. Phillips


A. Guildford


V. Perugini


S.T. Meikle

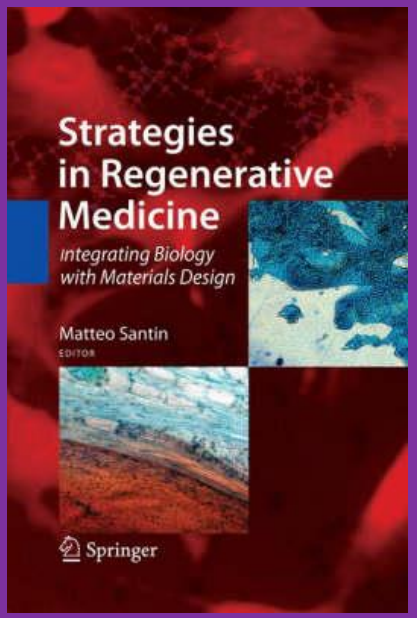

M Best

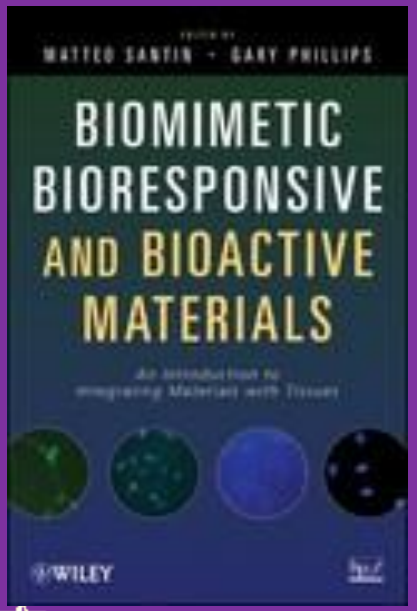

K. Ng


M. Verdenelli

AngioMatTrain
FP7-PEOPLE-2012-ITN

m.santin@brighton.ac.uk





University of Brighton

JOIN US!

- Active member: 130 Euros;
- Junior member: 25 Euros;
- Senior member: 50 Euros;
- Lab membership: 230 Euros;
- Corporate member: 300 Euros

European Society for Biomaterials

www.esbiomaterials.eu

@ESBPresident

European Society for Biomaterials

6th China-Europe Symposium on Biomaterials in Regenerative Medicine

CESB2017

Porto Portugal

May 21-24 2017