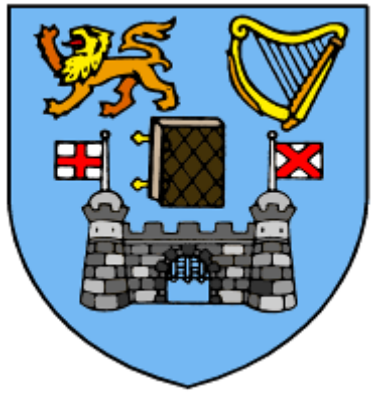
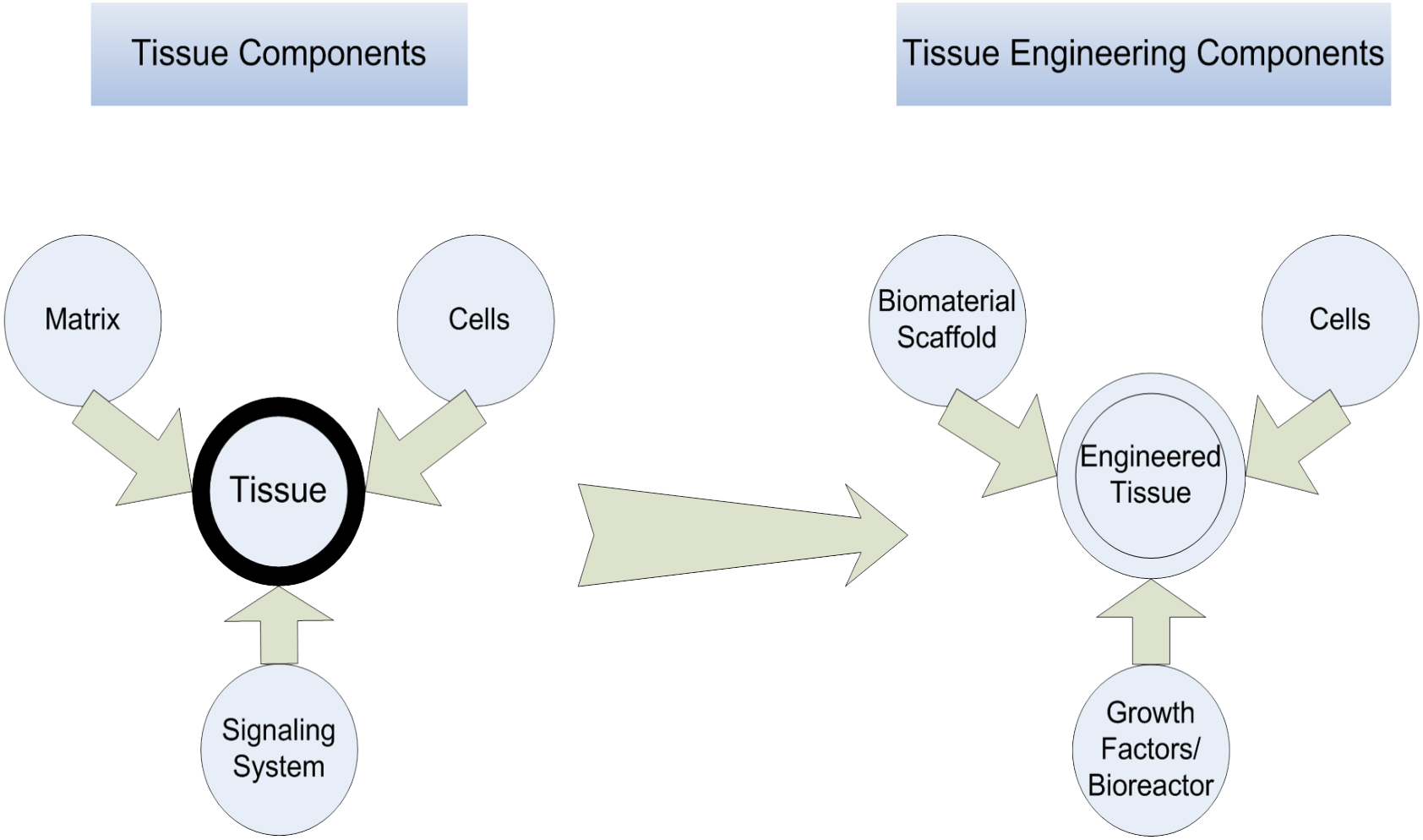


Tissue engineering scaffolds for cell and gene delivery

Prof. Fergal J. O'Brien, PhD

Dept. of Anatomy, Royal College of Surgeons in Ireland
Trinity Centre for Bioengineering, Trinity College Dublin

Tissue Engineering Triad



O'Brien Lab: Tissue Engineering Focus

- Biomaterial scaffolds
  - Collagen-glycosaminoglycan (tailored)
  - Collagen-ceramic composites

- Cells
  - Bone: osteoblasts, osteocytes
  - Cartilage: chondrocytes
  - CV: cardiomyocytes, smooth muscles cells, endothelial cells
  - Bone marrow derived mesenchymal stem cells
  - Amniotic fluid-derived stem cells
  - Cardiac stem cells
  - Limbal stem cells

- Cell signalling
  - Bioreactors & Mechanobiology
    - role of biophysical stimuli in regulating stem cell differentiation and tissue formation
  - Growth factors

  - Genes

Angiogenesis

+VEGF
+Ephrin- B2
+ Co-cultures
+ Hypoxia
+ Flow perfusion

Funded by: HRB, SFI, HEA PRTLI, Enterprise Ireland & European Research Council (FP7)

Scaffolds/Construct: Properties

- Biodegradable materials serving in a transitional role
  - Degradable (non- toxic)

- Biocompatibility
  - -immune response
  - -bioactive rather than biotolerable
  - -chemical composition, ligand density

- Appropriate mechanical properties
  - -replicate tissue at injured site
  - -clinical environment - ease of handling

- Pore architecture
  - 3D structure - open, interconnected, homogeneous pores
  - suitable for cell migration
  - allow for nutrient and waste exchange
  - architecture to support vasculature
  - incorporation of new tissue

Scaffold Biomaterials

- Synthetic biomaterials
- Natural biomaterials
- Composites

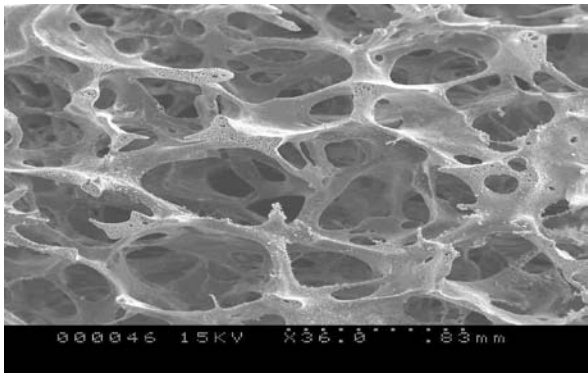
Synthetic Biomaterials

- Biodegradable polymers: poly(**α**-hydroxy)esters (PGA, PLA, PLGA)

-Polycaprolactones, polycarbonates, polyanhydrides, polyfumarates, polyorthoesters

-Ceramics/glasses: HA, ß-TCP, bioactive glasses

PLGA scaffold
(Holy *et al.*)

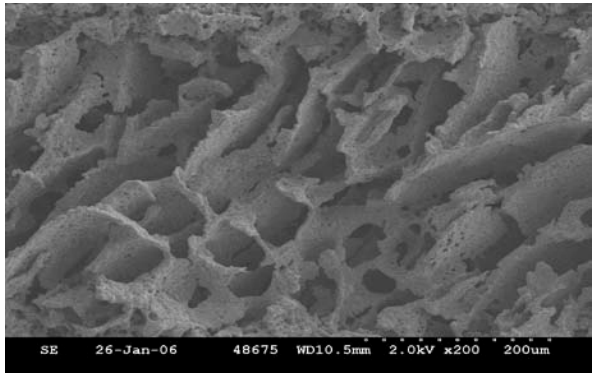


- **Advantages:**

  biodegradable, processing, pore architecture

  mechanical properties

HA scaffold
(Buckley *et al. 2010*)



- **Disadvantages:**

  inflammation, degradation rates, loss of cell function

Natural Biomaterials

Proteins: collagen, fibrin, elastin

Polysaccharides: alginate, chitosan, GAGs

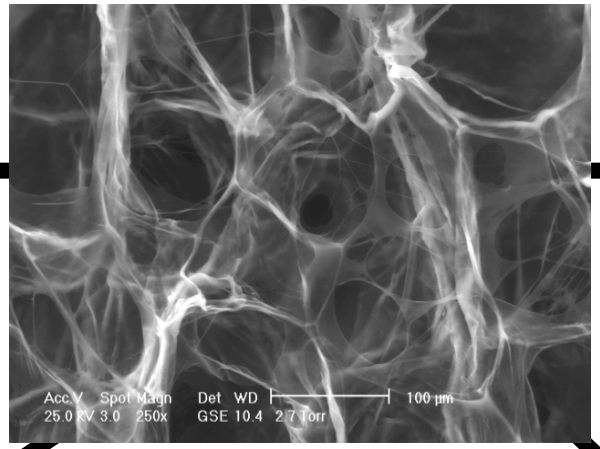
- Advantages: cell attachment, natural function, remodelling, less inflammation
- Disadvantages: mechanical properties, stability, processing

Collagen-based Scaffolds

O'Brien et al. Biomaterials 2004; 25: 1077-1086, Biomaterials 2005; 26: 433-441.
Technology & Healthcare 2007; 13: 1-15.

Collagen-based Scaffolds

Excellent Biocompatibility



Homogeneous Pore Size

50µm - 500µm

High Porosity

~ 99.5%

Biodegradable

Non- Toxic degradation products

O'Brien Lab

Optimised composition and pore structure for specific applications

Improved mechanical properties

Bone Repair

- 2.5 million bone grafts procedures annually worldwide
  - Non-union fractures
  - Implants, plates & screws
  - Spinal fusion

  } orthopaedic

  - Regeneration following injury, infection & disease eg: osteosarcoma, osteomyelitis
  - Reconstructive surgery eg: maxillofacial and dental applications

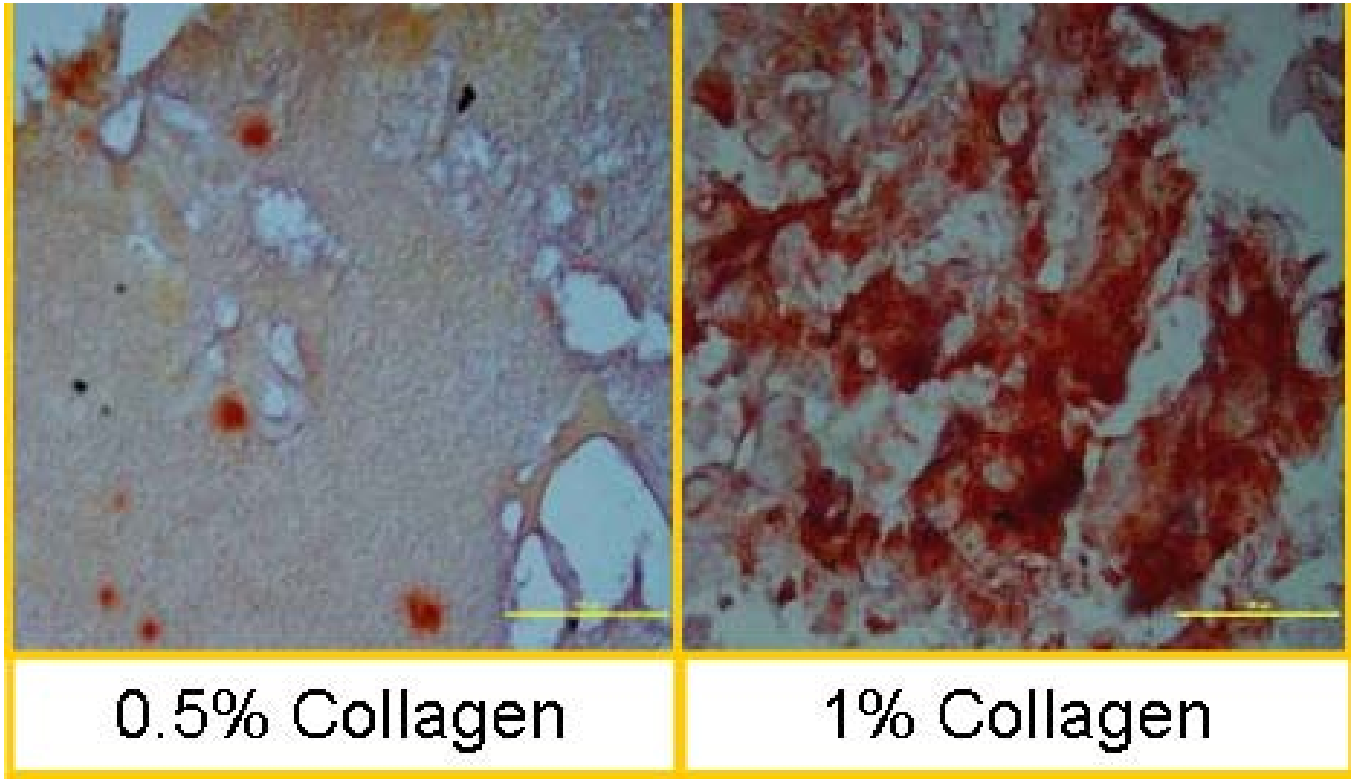
- Second most transplanted tissue after blood

- Current clinical treatment:

  Autografts: lack of tissue & donor site morbidity
  Allografts: lack of donors & risk of infection

  Bone graft substitutes and other approaches: no ‘gold standard’

Optimised Composition for Osteogenesis



Tierney, C.M.; Jaasma, M.J. & O'Brien, F.J. (2009) Osteoblast activity on collagen-GAG scaffolds is affected by collagen and GAG concentration. Journal of Biomedical Materials Research: Part A, 91A(1):92-101.

Tierney, C.M. et al. (2009) The effect of collagen concentration and crosslink density on biological, structural and mechanical properties of collagen-GAG scaffolds for bone tissue engineering. Journal of the Mechanical Behavior of Biomedical Materials (2): 202-209.

Scaffold Pore Size

- Mean pore diameter of collagen-GAG scaffolds determined by the process of ice crystal formation during freeze-drying of the slurry

Why is pore size important?

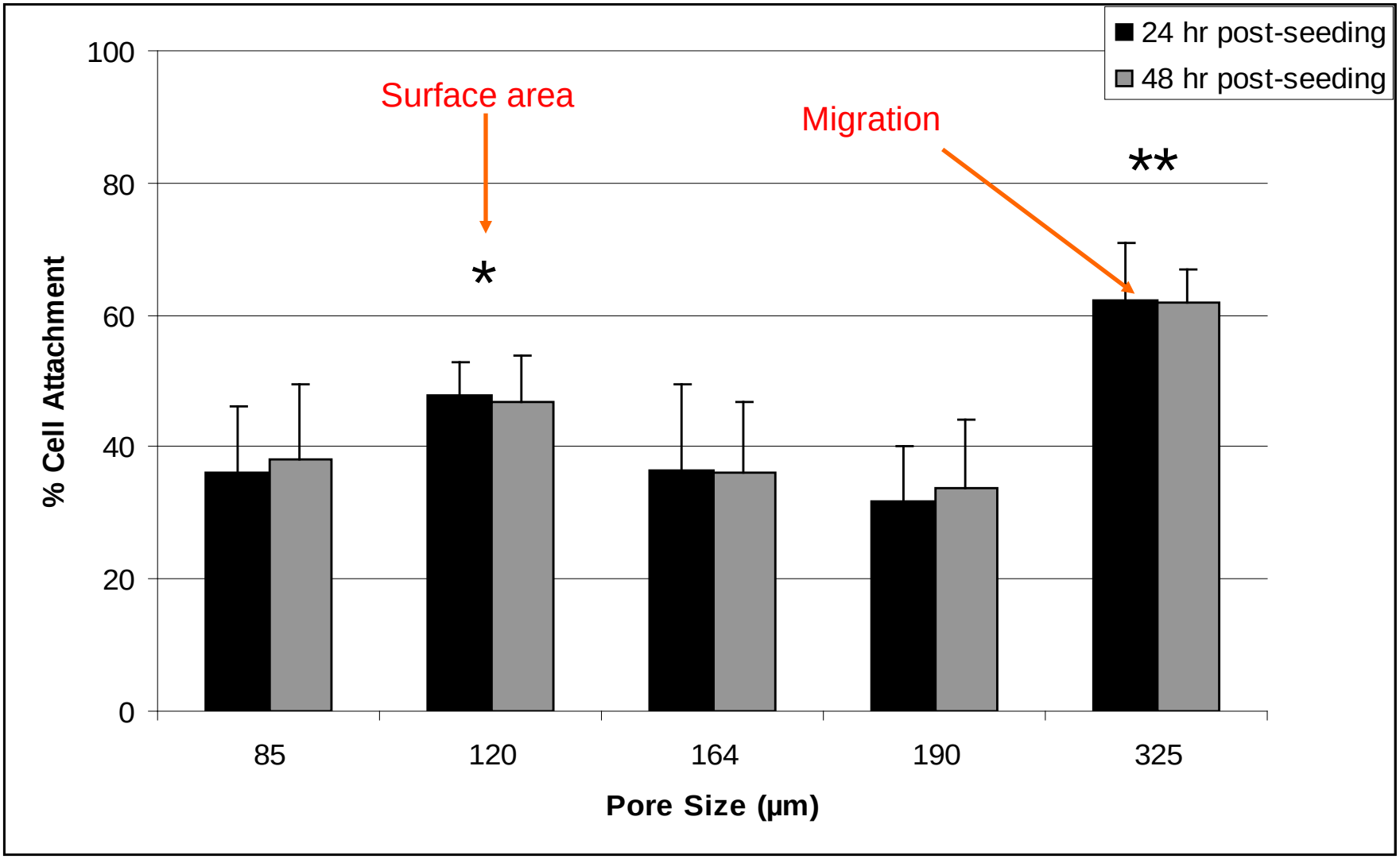
Pores are too small:

- cells cannot migrate
- limit diffusion of nutrients
- limit removal of waste products
- limit vascularisation

Pores are too large:

- decrease in ligand density
- limit cell attachment

Effect of Pore Size on Cell Behaviour



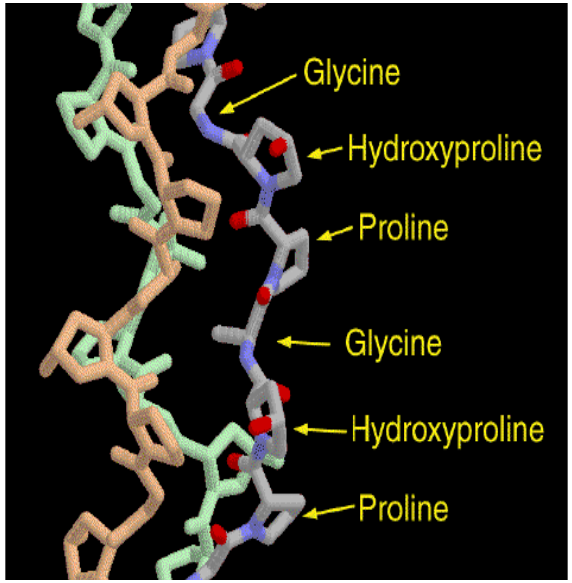
O'Brien, F.J.; Harley, B.A.; Yannas, I.V.; Gibson, L.J. (2005). *Biomaterials 26: 433-441.*

Murphy, C.M.; Haugh, M.G. and O'Brien, F.J. (2010) *Biomaterials* 31: 461–466.

Haugh, M.G.; Murphy, C.M. and O'Brien, F.J. (2010) *Tissue Engineering Part C* 16(5):887-94.

Optimised Mechanical Properties

- Stiffness
  - *In vivo* functionality
  - Surgical handling
- Crosslinking
  - Intra- and inter- helical crosslinks
  - Increase the modulus
  - Decrease the degradation rate

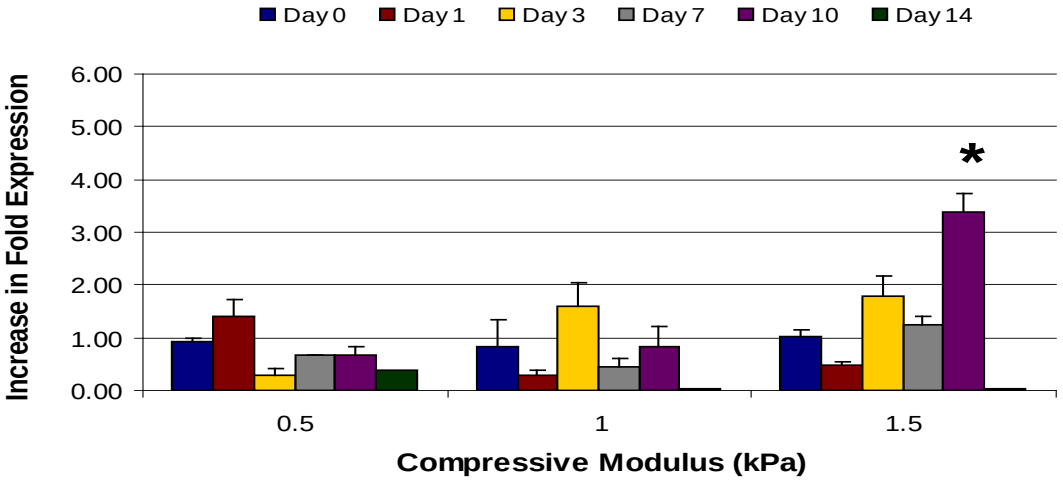


Triple helix

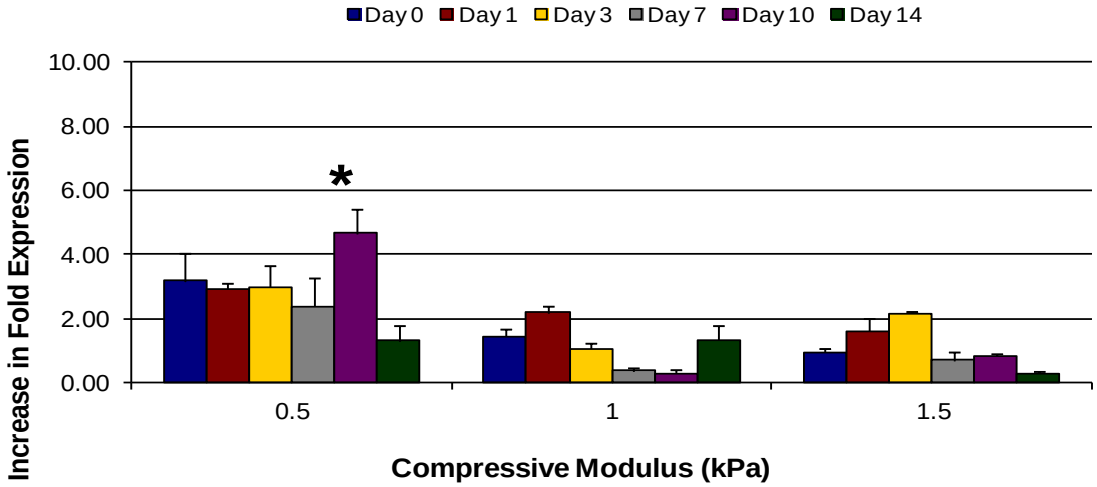
Haugh, M.G., Jaasma, M.J. and O'Brien, F.J. (2009) Effects of dehydrothermal crosslinking on mechanical and structural properties of collagen-GAG scaffolds. Journal of Biomedical Materials Research: Part A, 89A(2): 363-369.

Haugh et al (2011) Crosslinking and mechanical properties significantly influence cell attachment, proliferation and migration within collagen glycosaminoglycan scaffolds. Tissue Eng Part A. 7(9-10):1201-8.

Scaffold stiffness regulates MSC fate



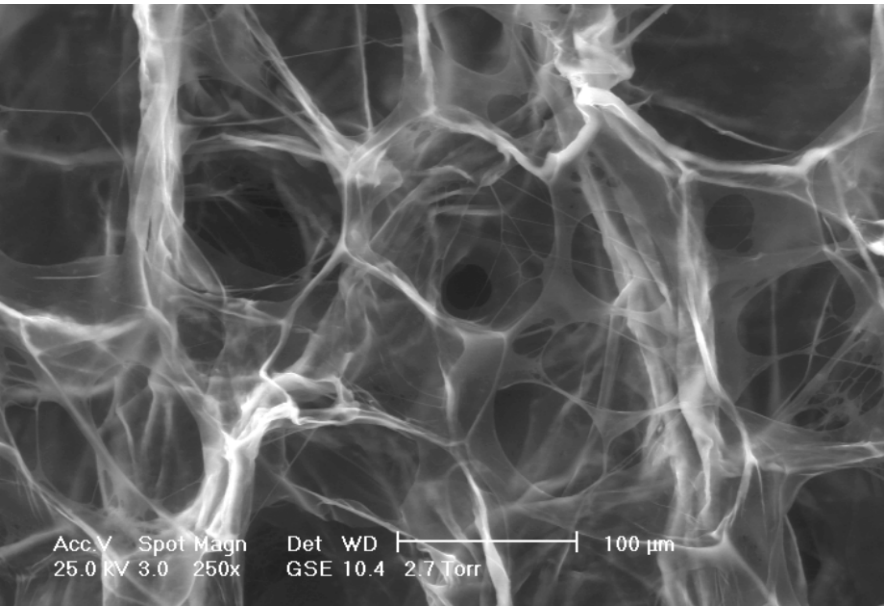
Osteogenic Lineage
RUNX2 Expression



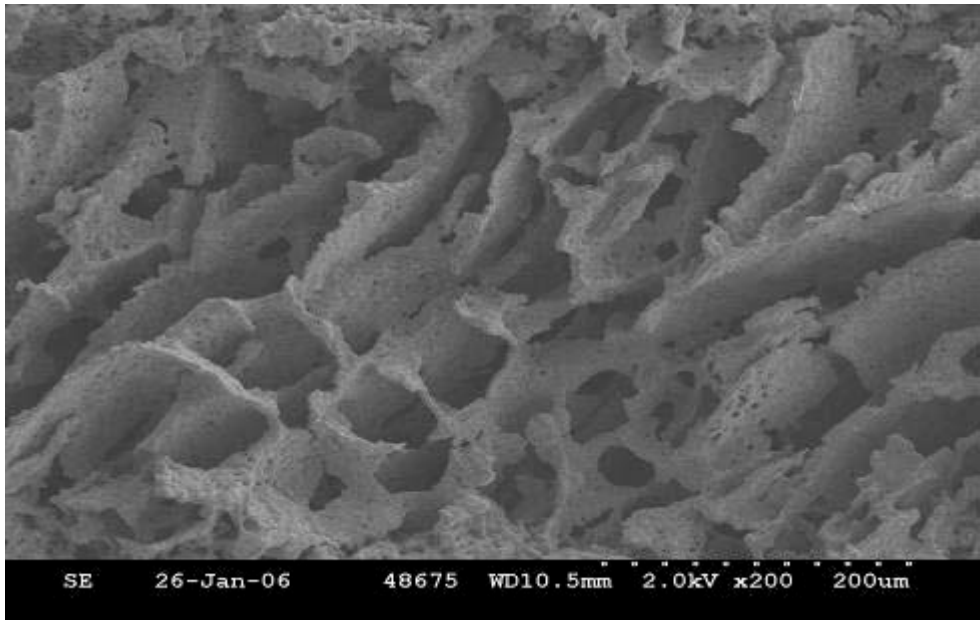
Chondrogenic Lineage
SOX9 Expression

Murphy et al. (2012) Mesenchymal stem cell fate is regulated by the composition and mechanical properties of collagen-glycosaminoglycan scaffolds. Journal of the Mechanical Behavior of Biomedical Materials 2012;11:53-62.

Composite Collagen-Ceramic Scaffolds



Collagen scaffold



Hydroxyapatite scaffold

- High stiffness
- High compressive strength

But

- Low porosity & permeability
- Low tensile strength
- Issues with degradation

SFI PIYRA 2004
SFI RFP 2006
Enterprise Ireland

Composite Collagen-Ceramic Scaffolds

(i) Collagen- HA (Drs. Niamh Plunkett & John Gleeson)

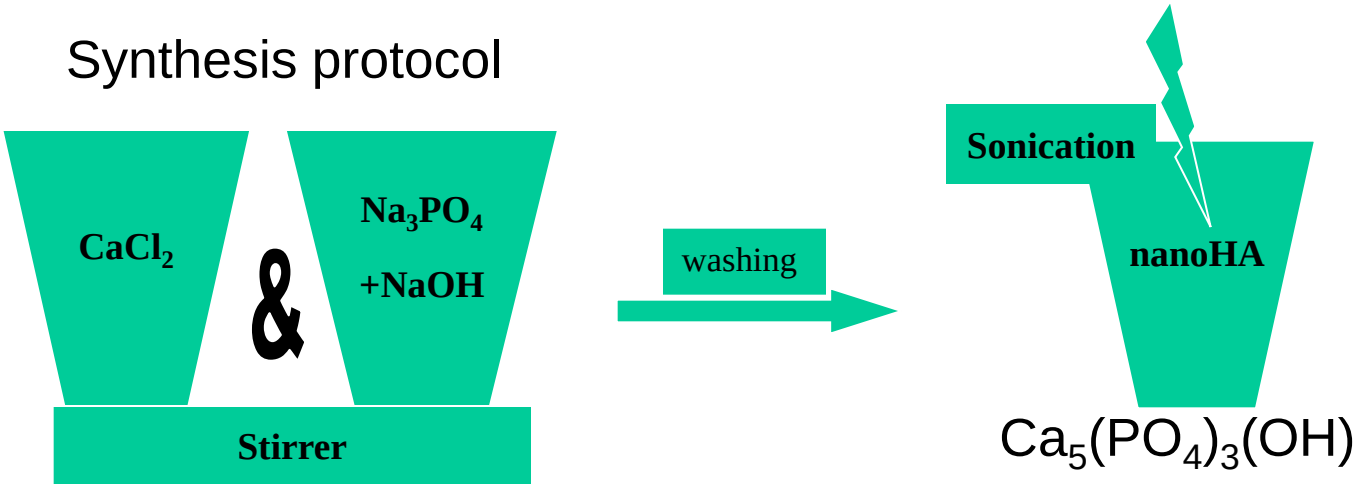
(ii) Collagen- Calcium Phosphate (Dr. Amir Al Munajjed)

(iii) Collagen- nano-HA (Dr. Grainne Cunniffe)

- Mechanical & Structural Analysis
- In vitro (cell) Analysis
- Pre-clinical (in vivo) Analysis

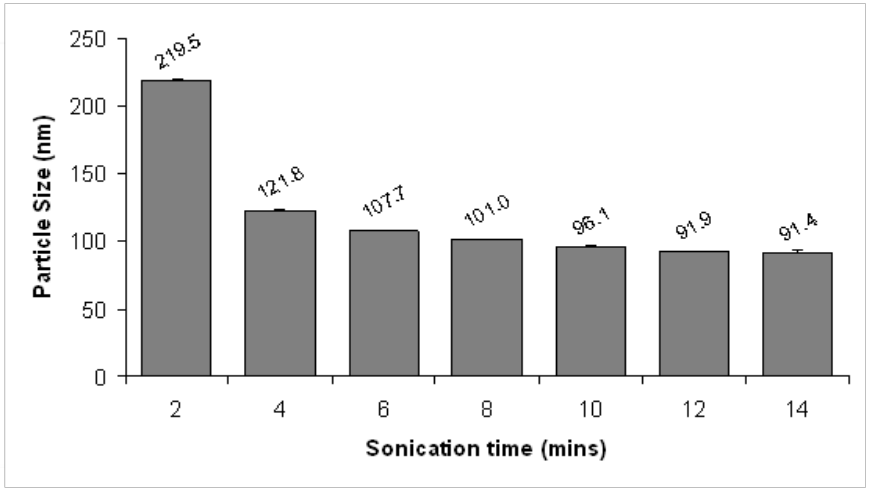
nHA synthesis

Synthesis protocol



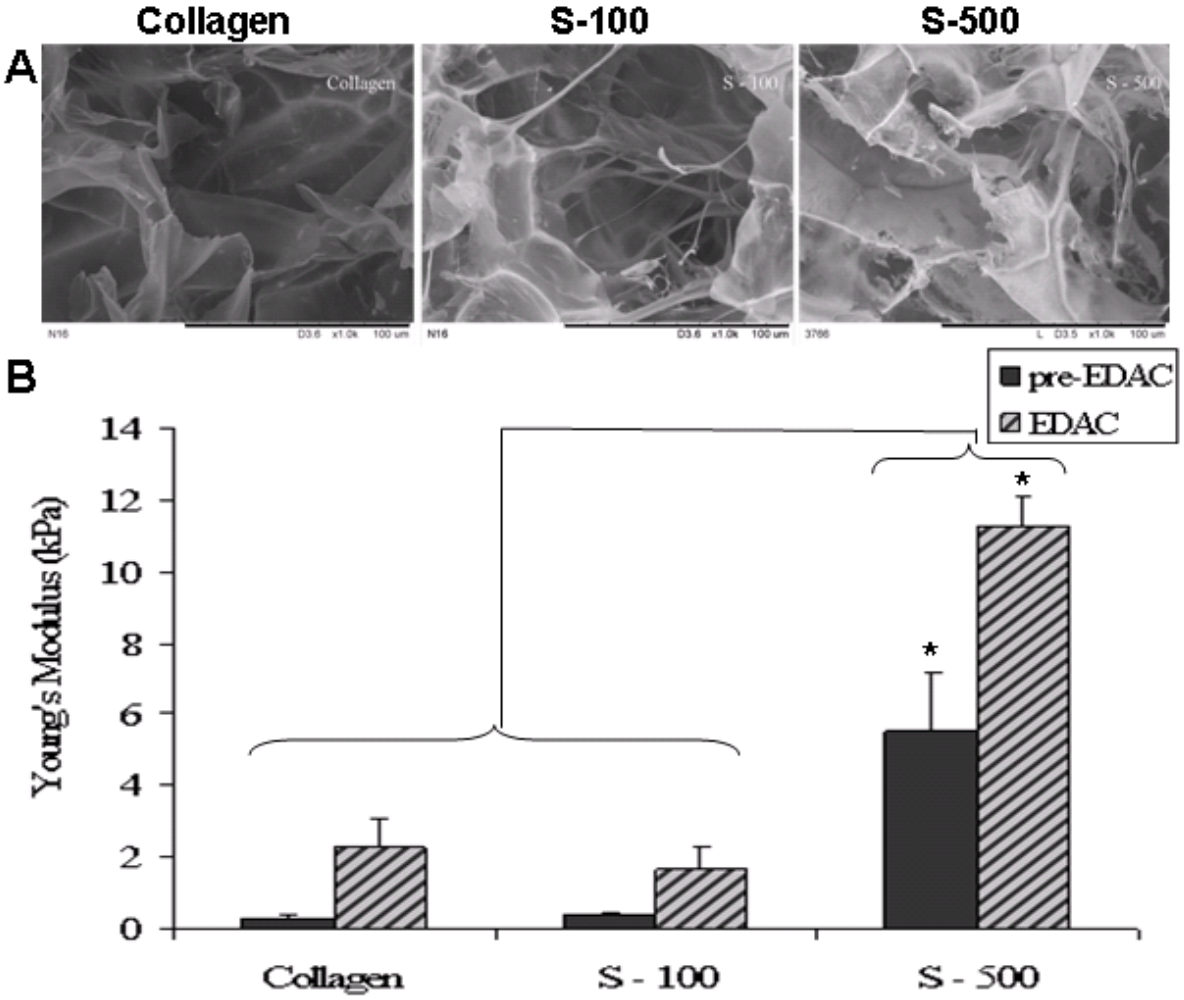
Parameters Optimised

Concentration of initial reactants
Reaction pH
- Order of reactant addition
- Use of dispersants– PVA and Darvan
- Use of sonication



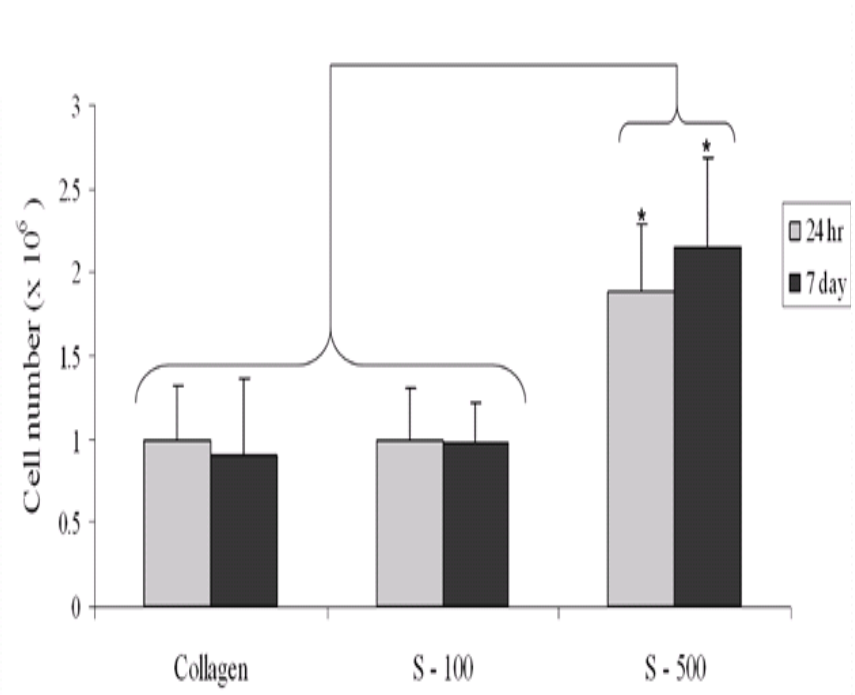
Cunniffe G. et al. (2010) Journal of Biomedical Materials Research: Part A. 95A (4):1142-1149.

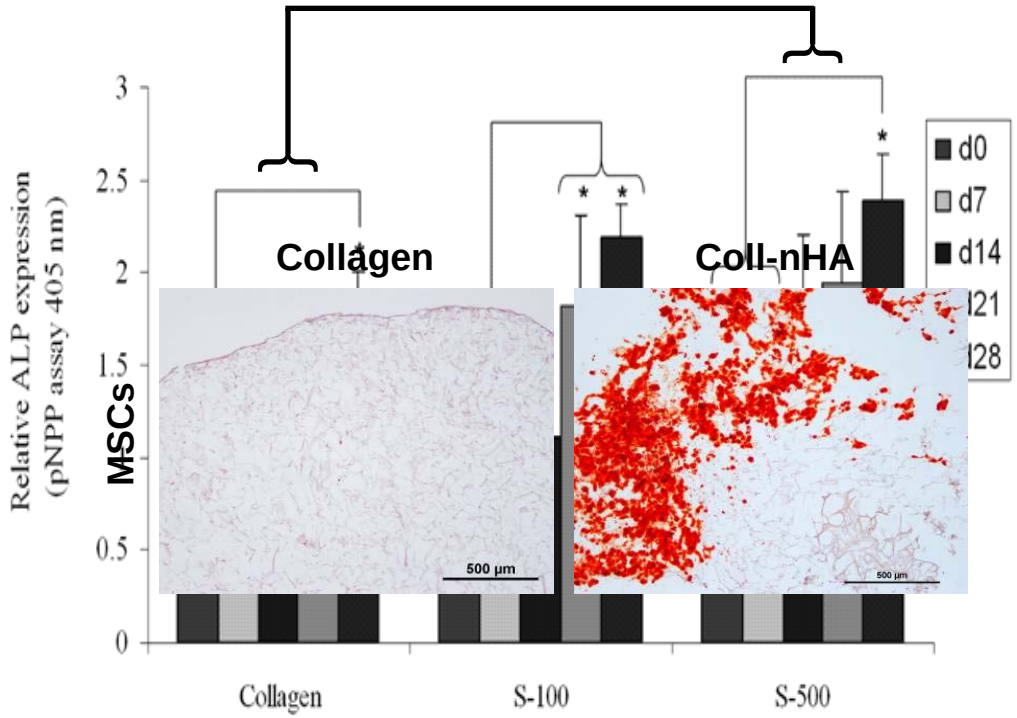
Effect of nano-HA addition & crosslinking on mechanical properties of collagen scaffolds



Cunniffe et al. (2010) Journal of Materials Science: Materials in Medicine 21(8): 2293- 2298.

Addition of nano-HA on cellular behaviour and osteogenesis



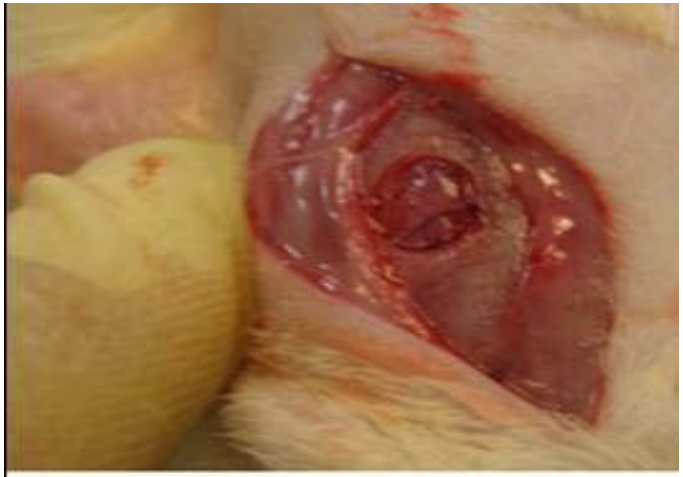


Increase in cell number

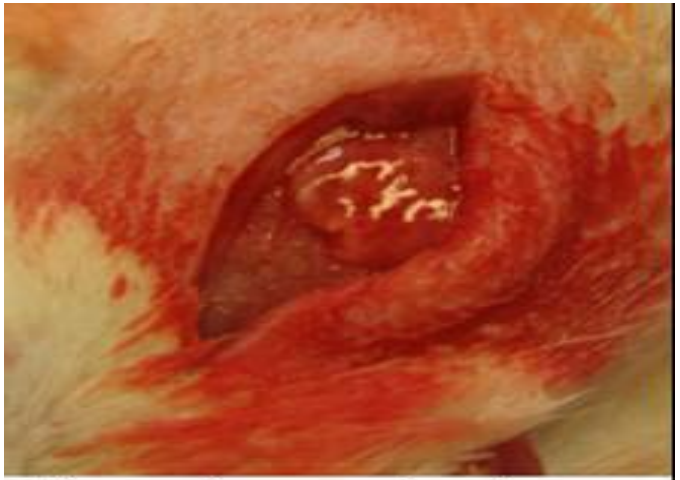
Increase in osteogenesis

Cunniffe et al. (2010) Journal of Materials Science: Materials in Medicine 21(8): 2293- 2298.

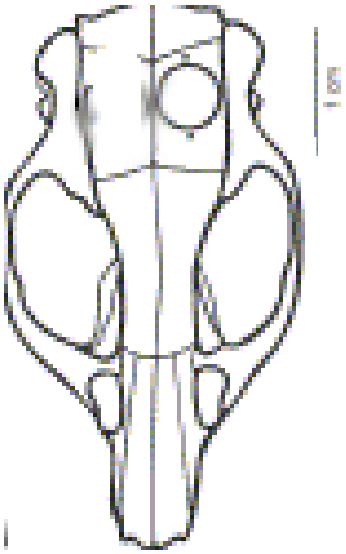
Assessment of in vivo healing



8mm calvarial defect



The graft occupying the defect

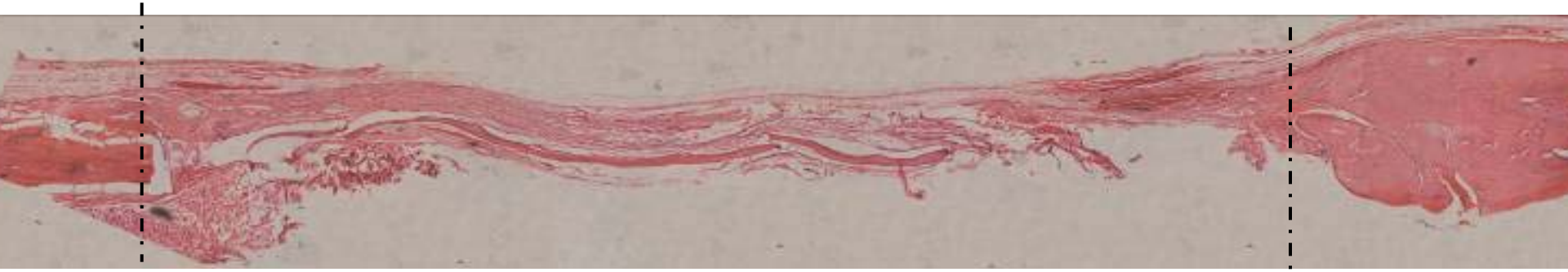


Lyons, F.; Al-Munajjed, A.; Kieran, S.; Toner, M. and O'Brien, F.J. (2010) The healing of bony defects by cell-free collagen-based scaffolds compared to stem cell-seeded tissue engineered constructs. Biomaterials 31(35):9232-43.

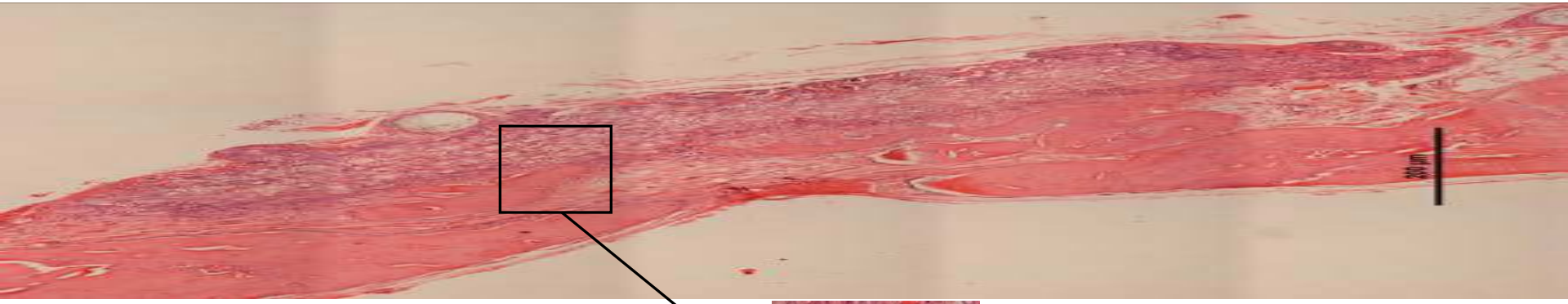
Alhag M. et al. (2011) Oral and Maxillofacial Surgery 15(1):31-9.

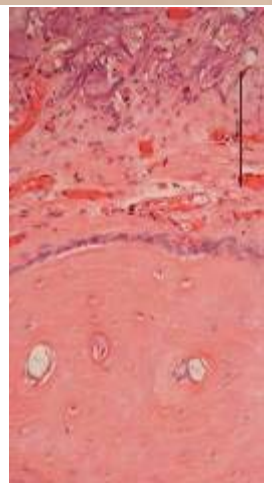
Alhag M. et al. (2012) Oral and Maxillofacial Surgery 16(1):47-55

Control (8 weeks)



Collagen-Ceramic (8 weeks) - Cell Free





Alhag M. et al. Oral and Maxillofacial Surgery (2011) 15(1):31-9.

Lyons F., Kieran S. et al. (2010) Biomaterials 31(35):9232-43.

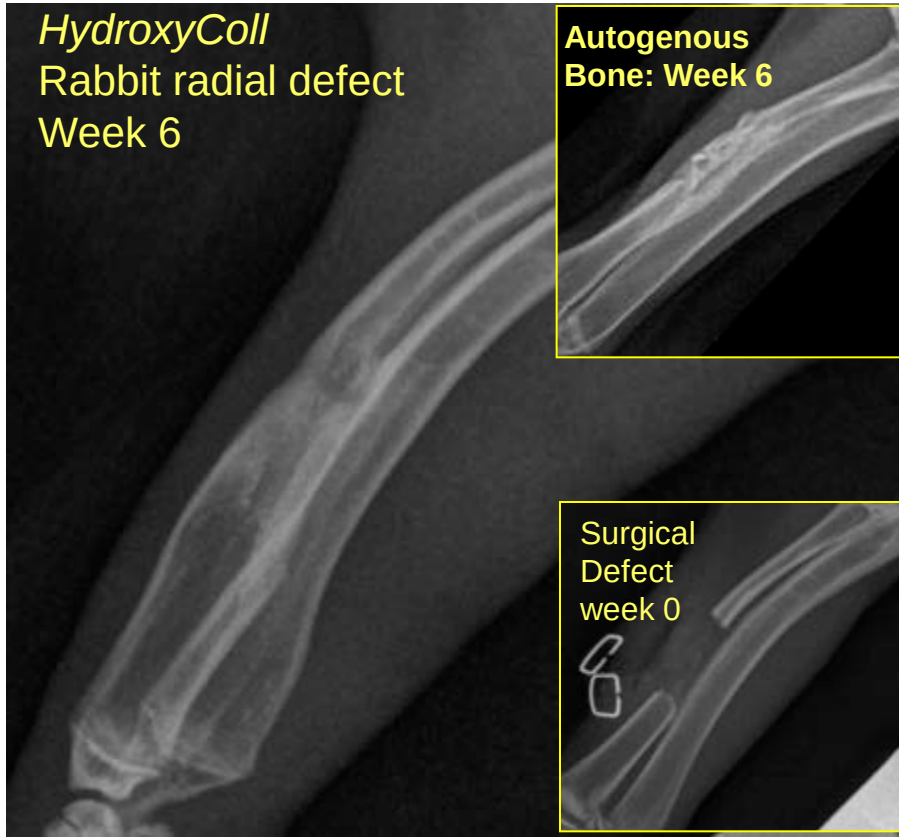
HydroxyColl

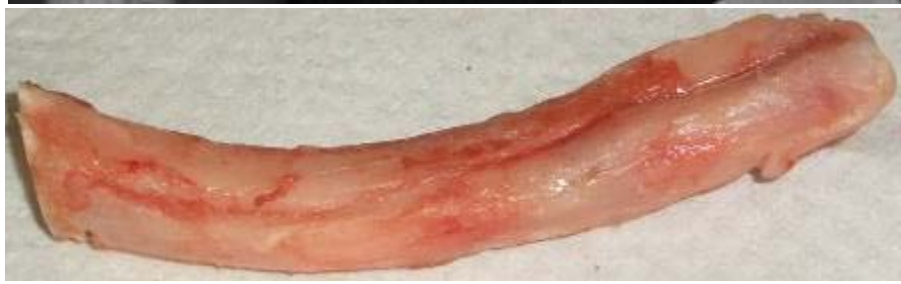
Bioengineering in Ireland 16 (2010) "*Best Overall Paper*"

RAMI Bronze Medal

CHA Scaffold

- Bone Tissue Regeneration
- Collagen-HA Scaffold
  - Patent filed (WO2008096334)
  - Undergoing regulatory approval process (FDA 510k /CE mark)





Clinical trials sooner than later

Gleeson JP, Plunkett NA, O'Brien FJ.
eCells and Materials (2010) 20: 218-230.

ENTERPRISE IRELAND
Transforming Irish Industry

Enterprise Ireland Commercialisation Fund
POC PC/2007/331 & CFTD TD/2007/112

SurgaColl

Surgical innovation delivered naturally

RCSI spin-out closes €2m investment

Elaine O'Regan

Company: SurgaColl
Done Deal: €2 million
Location: Cork
Staff: four
Established: 2010

Eric Reed, partner, Enterprise Equity; professor Fergal O'Brien, the Royal College of Surgeons in Ireland; and Dan Philpott, chief executive and co-founder of SurgaColl Technologies

SurgaColl, a spin-out of the Royal College of Surgeons in Ireland (RCSI), has closed a €2 million syndicated investment round involving AIB Seed Capital Fund, Harmac Medical Products, Enterprise Ireland and a number of private investors in Britain, France and Singapore. The AIB Seed Capital Fund, co-managed by Enterprise Equity Venture Capital, has completed a €500,000 equity investment in SurgaColl.

A tissue regeneration company based in the Rubicon Centre in Cork Institute of Technology, SurgaColl will use the funding to commercialise breakthrough tissue regeneration technology developed by the RCSI.

The multimillion-euro investment coincides with the licensing by SurgaColl Technologies of breakthrough RCSI technologies for the regeneration of human tissue.

The company was established in 2010 with VC backing to commercialise implantable orthopeadic products developed by RCSI's Tissue Engineering Research Group. The products use naturally derived materials for the regeneration of human tissue, including bone and cartilage.

"This investment reflects the confidence of our investors in the company and in its collagen-based platform technology.

"The funding will enable the company to launch its first product, HydroxyColl – an osteoinductive bone graft substitute – in the global orthopaedics market and to complete pre-clinical trials of a very promising cartilage regeneration product, ChondroColl," said Dan Philpott, chief executive and co-founder of SurgaColl Technologies.

The Royal College of Surgeons in Ireland is a not-for-profit organisation dedicated to improving human health through education, research and service.

Founded in 1784, originally to train surgeons, it provides education and training in the healthcare professions at both undergraduate and postgraduate level.

"The college is delighted to have completed the execution of intellectual property licence agreements and the set-up of a new Irish technology company, SurgaColl Technologies," said Dr Gearóid Tuohy, director, RCSI Technology Transfer Office.

"The investment by Irish and international investors will enable the SurgaColl team to deliver a number of key objectives in advancing the technologies to market. These achievements represent significant milestones for the college and its inventors, and further underscore RCSI's commitment and focus on the development of its research outputs for the benefit of patients, clinical care and industry."

Enterprise Equity Venture Capital jointly manages the AIB Seed Capital Fund of €53 million on a nationwide basis.

The fund was established in 2007 and invests in start-up and early-stage companies or enterprises based in Ireland, which have high growth potential. The fund's limited partners are AIB Bank and Enterprise Ireland.

The fund invests in companies operating in all areas of technology, as well as medical devices and medtech.

Clinical Need for Advanced Therapeutic

- In order to heal large defects in humans an extra agent may be required (i.e. cells/growth factors)

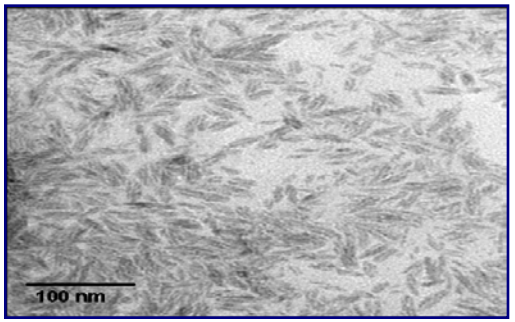
- Most commercially successful product to date:
  - Medtronic INFUSE™ bone graft substitute
  - Collagen sponge soaked with rhBMP-2
    - Problems with delivery, dosage and even cancer risk

- Gene-activated matrices incorporating the BMP-2 gene could provide a more efficient alternative
  - By implementing gene therapy, the protein can be released in a sustained and controllable manner

Gene Therapy

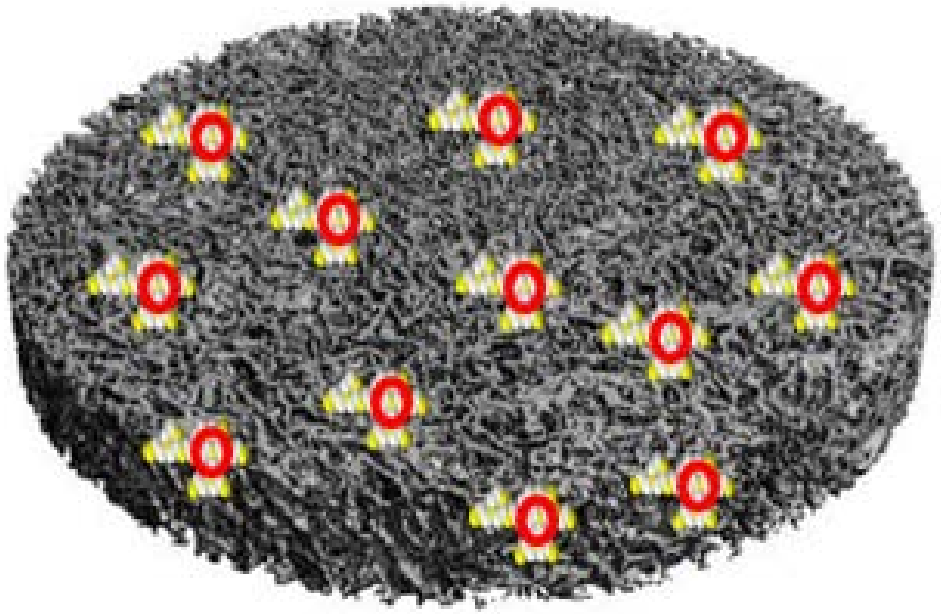
Efficient transfection system to introduce plasmid DNA into cells resulting in the expression of a specific gene (BMP-2)

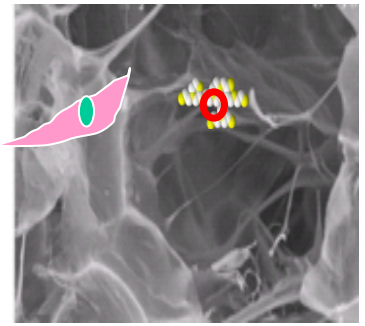
- Requires a delivery vector to transport the plasmid DNA into the cell
  - Viral
  - Non-viral
    - Lipid based technologies
    - PAMAM dendrimers
    - Chitosan
    - **PEI**
    - **Calcium phosphate (nHA particles)**

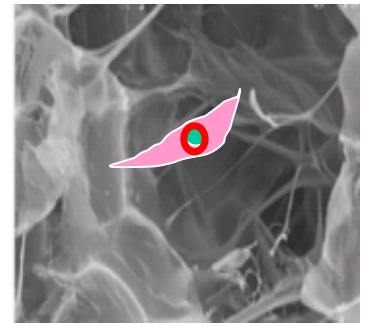


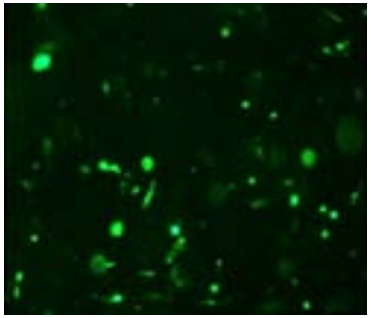
nHA particles

Gene-activated matrices (GAM) to promote enhanced vascularisation & bone healing









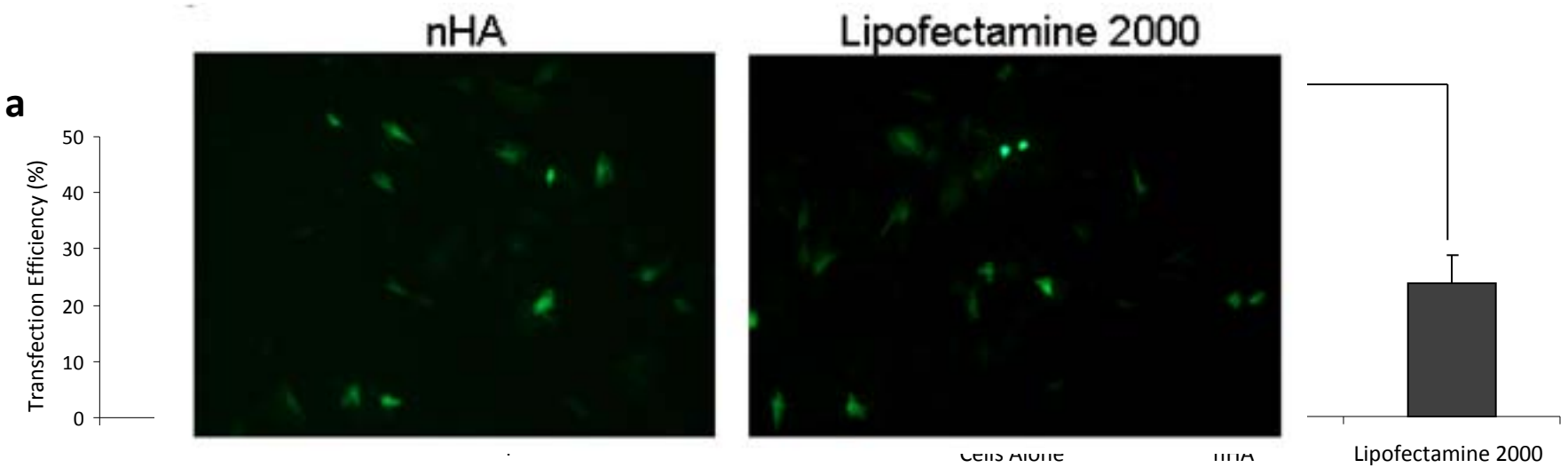
Dr. Caroline Curtin, Irene Mencia Castano
Erica Tierney, Rosanne Raftery

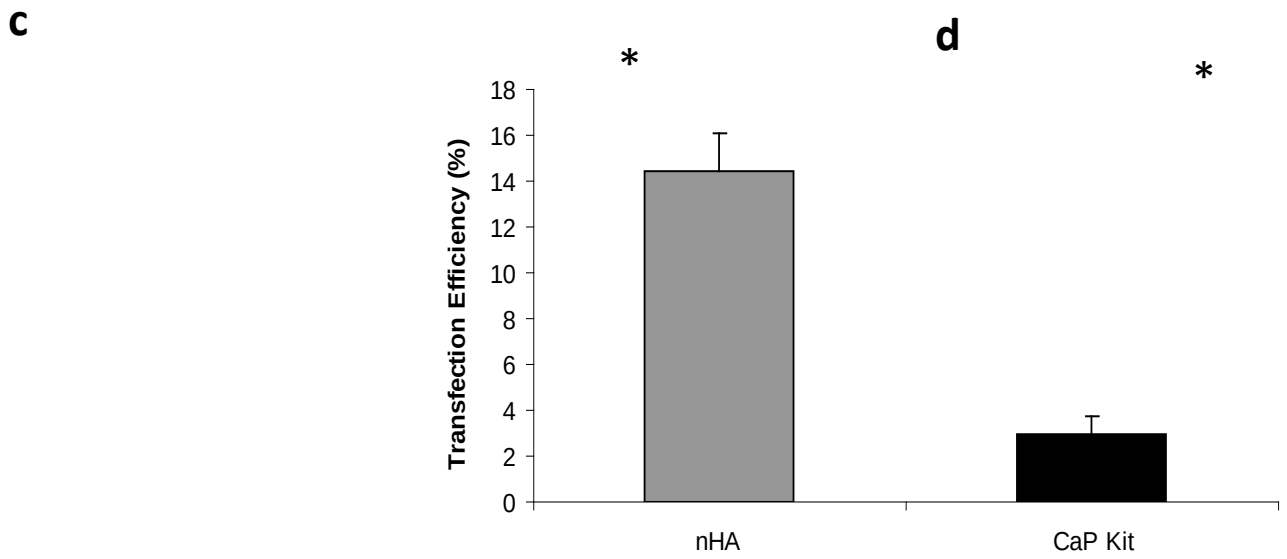
Tierney et al. (2012) Journal of Controlled Release 158(2):304-11

PEI-Gene-activated Scaffold: transfected human MSCs on coll-nHA scaffold 21 days post-seeding

SFI RFP 2011-2015

Nano-HA as non-viral transfection vectors in MSCs





Dr. Caroline Curtin & Dr. Frank Lyons

Gene-activated scaffolds: nano-HA particles as non-viral delivery vectors

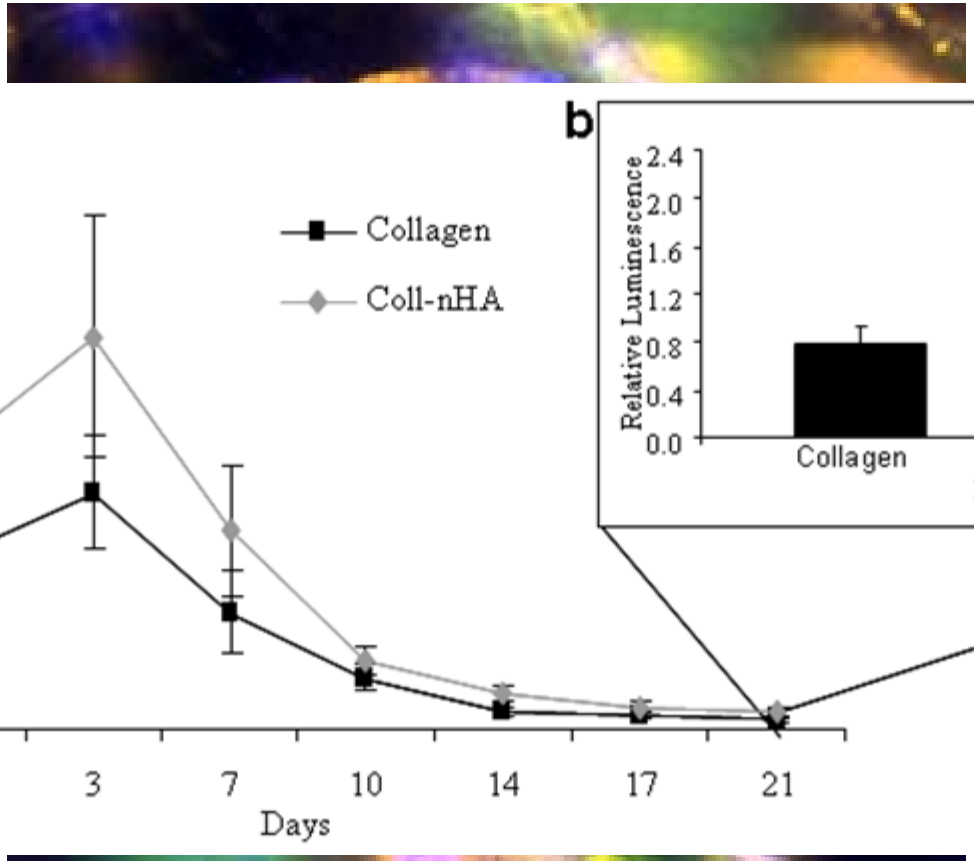


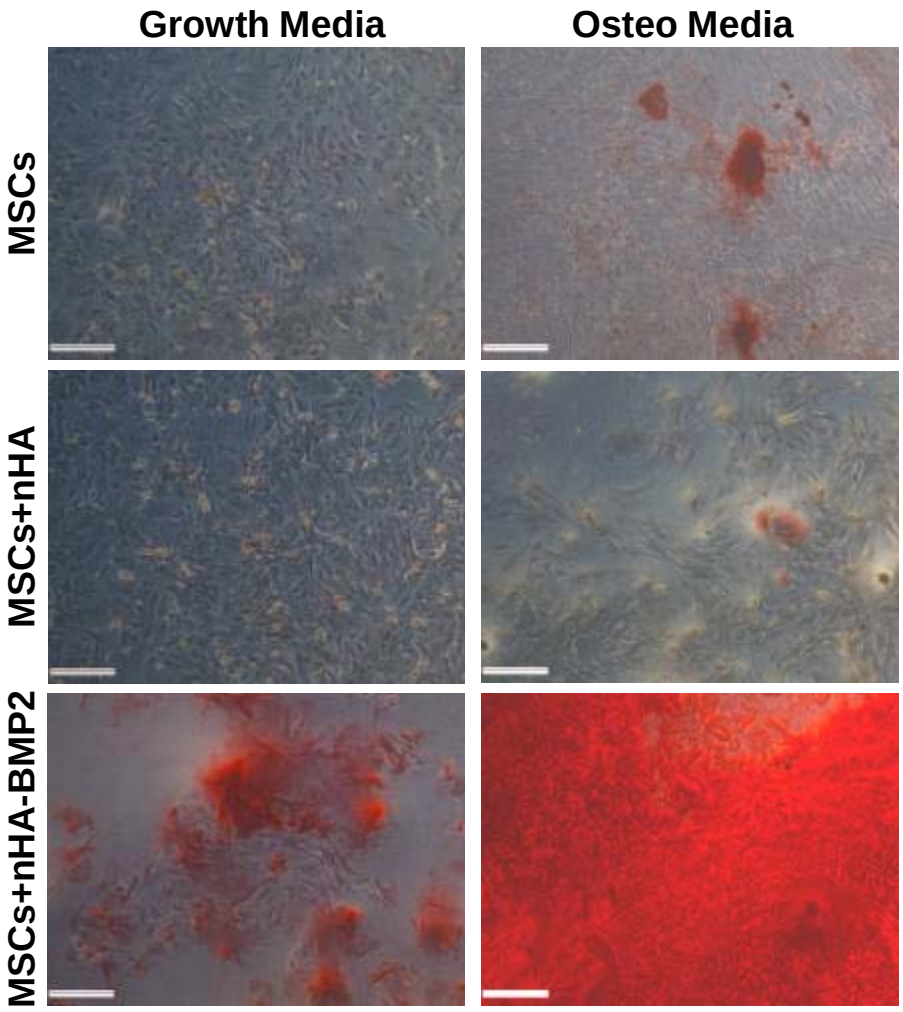
Image of GFP-transfected MSCs (green) on composite coll-nHA scaffold

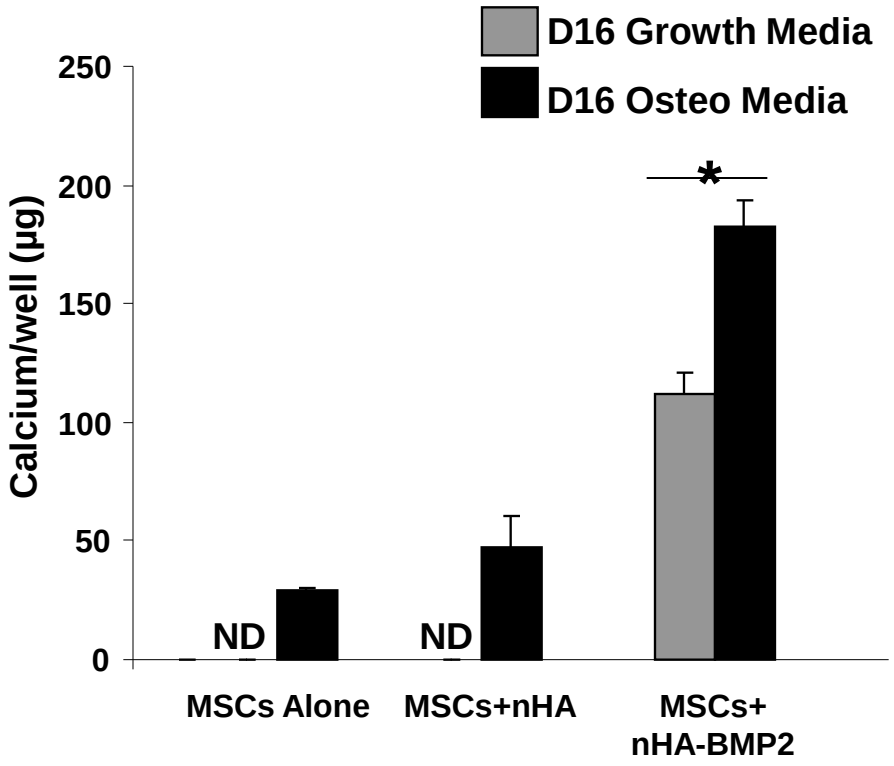
Sustained gene expression on composite scaffolds

Curtin C, Cunniffe G et al. (2012)

SFI RFP 2011-2015

Enhanced MSC osteogenesis following nHA BMP2 transfection





Curtin CM et al .(2012) Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation. Advanced Materials 2012; 24(6):749-54

Summary: Particles & Scaffold

- Reproducible method for synthesis of non-aggregating nanoHA particles (<100nm) established

- A collagen-nHA scaffold has been developed:
  - High porosity (>99%)
  - Homogeneous distribution of nHA particles
  - Improved mechanical properties
    (38-fold vs collagen only following crosslinking)

- Biological performance:
  - Increased cell proliferation
  - Increased osteogenic differentiation
  - Increased calcium production

Summary: Gene Delivery

- Novel nHA particles act as efficient non-viral delivery vectors for MSC transfection
- Incorporating nHA particles in collagen-based scaffolds results in a gene-activated matrix (GAM)
- Enhanced osteogenesis was observed when BMP2 was overexpressed in MSCs using nHA transfection both in 2D and 3D cultures

In summary, bioactive coll-nHA-BMP2 scaffolds have demonstrated **significant osteogenic capabilities** and potential for bone regeneration

Science Foundation Ireland
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