Recombinant Materials: Materials for the post-oil age

J. Carlos Rodríguez Cabello
G.I.R Bioforge. Uva-Ciber-BBN

Universidad de Valladolid
Valladolid (SPAIN)
Recombinamers: Recombinant Protein Polymers
Some Advantages of Recombinant Protein Polymers:

- They are able to incorporate any simple or complex function present in natural proteins.

- They are able to show any other amino-acid-based functionality of scientific or technological interest that evolution has not been selected in natural proteins.

- Absolute control in the polymer composition. That includes total absence of polydispersity and randomness.

- Huge number of possible compositions.

- They can be really complex in sequence, while an increase in complexity does not imply an increase in production costs.

- They are environmentally clean from production to waste.

- They are produced exclusively from biomass (do not require oil-derived materials).

...
Recombinant Technology:

- Direct Functionality given by (Bio)active peptide domains
- Functionality given by Nanometric design and control of Macromolecular composition

Holistic functionality by designing precise molecular architectures

Selected Examples:

- Injectable Hydrogels for Regenerative Medicine
- Smart Surfaces for Cell/Cell-sheet Harvesting
- Hierarchical morphogenesis
(VPGIG): Elastin pentapeptide.
(VPGKG): Elastin like pentapeptide modified to include the amino-acid K for cross-linking purposes.

(Mechanical Properties)
Extreme Biocompatibility
Smart and Self-assembling behaviour

Specific and Efficient Cell Attachment functionality

Adequate bioresobability and positive “Side Effects”

Regenerative Medicine: Material Designs

(VPGIG)_2 (VPGKG) (VPGIG)_2–(BIOACTIVE DOMAIN) (VPGIG)_2 (VPGKG) (VPGIG)_2 (VGVAPG)_3

(AXVGRGDSPASS): RGD loop of human fibronectin.
(QAASIKVAV): Neurite outgrowth peptide
(GTPGPQGIAGQRGVV): Osteoblast cell attachment
(EEIQIGHIPREDVYHLYP): Endotelial cell attachment
(DPGYIGSR): Laminin-derived non integrin-dependent cell attachment
(DDDEEKFLRRIGRG): Hydroxyapatite binding domain

(VGVAPG): Human elastin bioactive hexapeptide. This is target of ECM proteases and, as released, promotes cell movement, proliferation and angiogenesis.
Cell Therapy: Injectable systems

37°C
# Cell Therapy: Injectable systems

## HFF-1

<table>
<thead>
<tr>
<th>Time</th>
<th>Images</th>
</tr>
</thead>
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<td>1 day</td>
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<tr>
<td>15 day</td>
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<td>60 day</td>
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**MCS**

- ![Image](MCS_images)

**HUV ECs**

- ![Image](HUV_ECs_images)

**HFF-1**

- ![Image](HFF-1_images)
Bioactive Stents
Bioactive Stents
Cell Therapy: Injectable systems
Amphiphilic multiblocks

<table>
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<th>E50</th>
<th>I60</th>
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</table>

Hydrophilic block

T-Driven Hydrophobic block

BIOFORGE
3D Computed Tomography (CT)

Macroscopic Observation

Left Knees Hydrogel
Right Knees Hydrogel with MSC

Nuclear Magnetic Resonance (NMR)
-3D SPGR-

3D Computed Tomography (CT)
Full recovery of hyaline cartilage

Formation of ossification centers

Isogeneous groups of chondrocytes

Endochondral ossification and isogeneous groups of chondrocytes in the injured zone
### Quantitative Analysis

Histological scoring of cartilage repair according to modified Wakitani score.

<table>
<thead>
<tr>
<th>↓ CATEGORY</th>
<th># RABBIT</th>
<th>Hydrogel (left knees)</th>
<th>Hydrogel with MSC (right knees)</th>
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<tbody>
<tr>
<td>CELL MORPHOLOGY (0-4) *</td>
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<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
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<tr>
<td>MATRIX-STAINING (METACHROMASIA) (0-3) *</td>
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<td>1 2 1 1 2 2</td>
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<td>SURFACE REGULARITY (0-3) *</td>
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<td>THICKNESS OF CARTILAGE (0-3) *</td>
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<td>3 3 3 3 3 2</td>
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<tr>
<td>INTEGRATION WITH HOST ADJACENT CARTILAGE (0-2)</td>
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<td>2 2 1 2 2 1</td>
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<td>TOTAL (0-15)</td>
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<td>8 9 6 3 8 8</td>
<td>15 15 12 13 15 11</td>
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<td>AVERAGE (0-15)</td>
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<td>5.6</td>
<td>13.5</td>
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</table>

* Significant differences in the medians according to Kruskal-Wallis analysis.

Immunohistochemistry with mAb against a human mitochondria marker and DAPI stain.
Thermosensitive ELR Surfaces for Cell Harvesting

$37^\circ C$  
$75.8^\circ \pm 0.7^\circ$  
$65.5^\circ \pm 0.9^\circ$  
$T < T_t$
# Molecular Designs

<table>
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<tr>
<th>RECOMBINAMER</th>
<th>AMINO-ACID SEQUENCE + SCHEMATIC CARTOON OF THE MOLECULE</th>
<th>T&lt;sub&gt;c&lt;/sub&gt;/°C</th>
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<td>3K-RGD (1)</td>
<td>MGKKKP-(VPGVG)&lt;sub&gt;14&lt;/sub&gt;[(VPGIG)&lt;sub&gt;10&lt;/sub&gt;AVTGRGDSPASS(VPGIG)&lt;sub&gt;10&lt;/sub&gt;l&lt;sub&gt;2&lt;/sub&gt;(VPGVG)&lt;sub&gt;14&lt;/sub&gt;V</td>
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<td>RGD+24K (5)</td>
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<td>3K-RGD3 (6)</td>
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15 min at 10 °C

Graph showing % Adherent cells against Incubation Time (min) with conditions 37°C and 10°C. Graph indicates the effect of RGD on adherent cells across different temperatures and times.

Images show cell cultures at 37°C and 10°C with and without RGD.
Hierarchical Morphogenesis of a hybrid peptide amphiphilic/ELR system
Tube growing
ACKNOWLEDGEMENTS

Layered hierarchical structured scaffolds with injectable self setting bioactive gel for clinical bone tissue repair (FP7-NMP-2010-2.2.3)

Tissue in Host Engineering Guided Regeneration of Arterial Intimal Layer
FP7-Health-2011-278557

Development of biomaterial-based delivery systems for ischemic conditions; an integrated pan-European approach. FP7-People-2012-ITN

Engineering responsive and biomimetic hydrogels for biomedical therapeutic and diagnostic applications. MSCA-ITN-2014-ETN

Tailored elastin-like recombinamers as advanced systems for cell therapies in Diabetes Mellitus: a synthetic biology approach towards a bioeffective and immunoisolated biosimilar islet/cell niche. MMP-2014-642687
THANK YOU

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ciber-bbn

Technical Proteins Nanobiotechnology
EU Flag