RCP Publications (April 2019)

	Title	Authors	Year	Reference	Remarks / contact / context	Copy	Abstract
0	Secreted production of a custom-designed, highly hydrophilic gelatin in Pichia pastoris.	Werten MW, Wisselink WH, Jansen-van den Bosch TJ, de Bruin EC, de Wolf FA.	2001	Protein Engineering 14, 2001, 447-454	ATO-DLO	Available	A custom-designed, highly hydrophilic gelatin was produced in Pichia pastoris. Secreted production levels in single-copy transformants were in the range 3-6 g/l of clarified broth and purification to near homogeneity could be accomplished by differential ammonium sulfate precipitation. Despite the fact that gelatins are highly susceptible to proteolysis because of their unfolded structure, the recombinant protein was shown to be fully intact by SDS-PAGE, N-terminal sequencing, gel filtration chromatography and mass spectrometry. Owing to its highly hydrophilic nature, the migration of the synthetic gelatin in SDS-PAGE was severely delayed. Esterification of the carboxylic amino acid side chains resulted in normal migration. The high polarity of the synthetic gelatin also accounts for its negligible surface activity in water at concentrations up to 5% (w/v), as determined by tensiometry. Circular dichroism spectrometry showed that the non-hydroxylated gelatin did not form triple helices at 4 degrees C. The spectrum was even more representative of the random coil conformation than the spectrum of natural non-hydroxylated gelatins.
1	Recombinant gelatin hydrogels for the sustained release of proteins	Sutter, Siepmann, Hennink, Jiskoot	2007	Journal of Controlled Release, 119, (2007) 301-312	Utrecht University	Available	A recombinant gelatin (HU4) containing part of the amino acid sequence of the α1-chain of human type I collagen was used for preparing hydrogels for the sustained release of proteins. HU4 gelatin was modified with methacrylate residues for chemical crosslinking and gel formation. Methacrylated gelatins with degrees of substitution (DS; defined as fraction of methacrylate residues with respect to the total number of primary amines) of 0.24, 0.67, 0.82, and 0.97 were synthesized, and hydrogels with polymer volume fractions in the swollen state (υ2,s) between 0.01 and 0.14 were formed by radical polymerization. Mesh size (ξ) was ≥26 nm, as determined by dynamic mechanical analysis. Release of the incorporated model proteins lysozyme and trypsin inhibitor occurred by diffusion and was nearly complete. Protein diffusion coefficients in the gel were between 5.0×10−7 and 4.0×10−8 cm2 s−1, up to 100 fold lower than in water. Release under physiological conditions was effectively controlled by varying hydrogel mesh size and protein–gelatin charge interactions, which demonstrates that recombinant gelatins are a versatile class of biopolymers for the preparation of hydrogels for protein delivery. HU4 hydrogels were enzymatically degradable by human matrix metalloproteinase 1, which is an indication of their in vivo biodegradability.
2	Recombinant Gelatin Microspheres: novel formulations for tissue repair	Tuin, Kluijtmans, Bouwstra, Harmsen, van Luyn	2010	Tissue Engineering Part A, 16, 2010, 1811-1821	University Medical Centre Groningen (UMCG)	Available	Microspheres (MSs) can function as multifunctional scaffolds in different approaches of tissue repair (TR), as a filler, a slow-release depot for growth factors, or a delivery vehicle for cells. Natural cell adhesion–supporting extracellular matrix components like gelatin are good materials for these purposes. Recombinant production of gelatin allows for on-demand design of gelatins, which is why we aim at developing recombinant gelatin (RG) MSs for TR. Two types of MSs (50<Ø<100 mm) were prepared by crosslinking two RGs, Syn-RG, and the arginine-glycine-aspartate-containing Hu-RG. The MSs were characterized, and their tissue reaction and degradation in rats was examined. Histological analysis of the explants after 14 and 28 days in vivo also showed that Syn-RG was degraded slower than Hu-RG, which correlated with the in vitro degradation assay. Hu-RG explants displayed more cellular ingrowth (60% vs. 15% for Syn-RG at day 14), which was associated with extracellular matrix deposition and vascularization. The infiltrating cells consisted of mainly macrophages, part of which fused to giant cells locally, and fibroblasts. No differences were found in matrix metalloproteinase mRNA levels, whereas gelatinase activity was clearly higher in Hu-RG explants. In conclusion, the in vitro and in vivo results of these novel formulations pave the way for cell- and=or factor-driven TR by these RG MSs.
3	A new fluorescent imaging of renal inflammation with RCP	Nakamura, Tabata	2010	Journal of Controlled Release 148, 2010, 351–358	Tabata	Available	The objective of this study is to design a fluorescent imaging agent with R-Gel, one of the recombinant polymers (RCP), for renal inflammation. The R-Gel based on human type I collagen has multiple Arg–Gly–Asp (RGD) motifs which are ligands for some types of integrin receptors on the cell surface. After intravenous administration of R-Gel labeled by Cy7 of a fluorescent dye to three animal models of nephritis mousse, interstitial nephritis (by using UUO model mice), glomerulonephritis (HIGA mice), and ischemia–reperfusion injured kidney (I/R mice), the extent of fluorescent

							imaging at the renal inflammation was assessed. The Cy7-labeled R-Gel was accumulated in the inflammation site to a significantly greater extent than in the normal one at 24 h after administration. The renal pattern of fluorescent imaging was similar to that of administration anti-Mac1 antibody. Taken together, it is conceivable that the R-Gel was targeted to macrophages infiltrated into the inflammation site of kidney.
4	Hyaluronic acid-recombinant gelatin gels as a scaffold for soft tissue regeneration	Tuin, Zandstra, Kluijtmans, Bouwstra, Harmsen,v an Luyn	2012	European Cells and Materials, 24, 2012, 320-330	UMCG	Available	An array of different types of hyaluronic acid (HA)- and collagen-based products is available for filling soft-tissue defects. A major drawback of the current soft-tissue fillers is their inability to induce cell infiltration and new tissue formation. Our aim is to develop novel biodegradable injectable gels which induce soft tissue regeneration, initially resulting in integration and finally replacement of the gel with new autologous tissue. Two reference gels of pure HA, monophasic HA-1 and micronised HA- 2, were used. Furthermore, both gels were mixed with recombinant gelatin (RG) resulting in HA-1+RG and HA-2+RG. All gels were subcutaneously injected on the back of rats and explanted after 4 weeks. Addition of RG to HA-1 resulted in stroma formation (neovascularisation and ECM deposition) which was restricted to the outer rim of the HA-1+RG gel. In contrast, addition of RG to HA-2 induced stroma formation throughout the gel. The RG component of the gel was degraded by macrophages and giant cells and subsequently replaced by new vascularised tissue. Immunohistochemical staining showed that the extracellular matrix components collagen I and III were deposited throughout the gel. In conclusion, this study shows the proof of principle that addition of RG to HA-2 results in a novel injectable gel capable of inducing soft tissue regeneration. In this gel HA has a scaffold function whereas the RG component induces new tissue formation, resulting in proper vascularisation and integration of the HA-2+RG gel with the autologous tissue.
5	Development of recombinant collagen-peptide-based vehicles for delivery of adipose-derived stromal cells (ADSC)	Parvizi, Plantinga, van Spreuwel-Goossens, van Dongen, Kluijtmans, Harmsen	2016	J Biomed Mater Res A. 2016 104(2), 503-16.	UMCG, BMM	Available	Stem cell therapy is a promising approach for repair, remodeling and even regenerate tissue of otherwise irreparable damage, such as after myocardial infarction (aMI). A severe limitation of cardiac stem cell therapy is the generally poor retention of administered cells in the target tissue. In tissue repair the main mode of action of adipose tissue derived stem cells (ADSC) is the production of various growth factors, cytokines, anti-inflammatory and anti-apoptotic factors that together augment repair, remodeling, and regeneration. In this experiment, we used recombinant collagen peptide (RCP) with additional integrin-binding motives and different crosslinkers. Formulated as 50–100 lm microspheres with bound ADSC, we hypothesized that this would improve ADSC retention and function. Crosslinking was performed with chemical crosslinkers (EDC and HMDIC) at high and low concentrations or by thermal treatment (DHT). ADSC adhesion, proliferation, apoptosis/necrosis, and gene expressions in two-dimensional and three-dimensional were analyzed. In addition, the effect of ADSC conditioned medium (ADSC-CM) on proapoptotic/sprouting HUVEC was examined. Our results show that all materials support cell adhesion in short time point, however, EDC-High crosslinker induced ADSC apoptosis/ necrosis. Gene expression results revealed lower expression of proinflammatory genes in chemical crosslinked materials, despite EDC-High the proinflammatory genes expressions were similar or higher than TCPS. In addition, cultured ADSC on DHT crosslinked RCP showed a proinflammatory phenotype compared to TCPS. Sprouting assay results confirmed the protective effect of ADSC-CM derived from TCPS and HMDIC-High crosslinked RCP proapoptotic HUVEC. We conclude that ADSC adhere to the materials and maintain their therapeutic profile
6	Biomimetic mineralization of recombinant collagen type I derived protein to obtain hybrid matrices for bone regeneration	Ramírez-Rodríguez, Delgado-López, Iafisco, Sandri, Sprio, Tampieri	2016	J. Struct. Biology, 196 (2016), 138-146	BioInspire	Available	Understanding the mineralization mechanism of synthetic protein has recently aroused great interest especially in the development of advanced materials for bone regeneration. Herein, we propose the synthesis of composite materials through the mineralization of a recombinant collagen type I derived protein (RCP) enriched with RGD sequences in the presence of magnesium ions (Mg) to closer mimic bone composition. The role of both RCP and Mg ions in controlling the precipitation of the mineral phase is in depth evaluated. TEM and X-ray powder diffraction reveal the crystallization of nanocrystalline apatite (Ap) in all the evaluated conditions. However, Raman spectra point out also the precipitation of amorphous calcium phosphate (ACP). This amorphous phase is more evident when RCP and Mg are at work, indicating the synergistic role of both in stabilizing the amorphous precursor. In addition, hybrid matrices are prepared to tentatively address their effectiveness as scaffolds for bone tissue engineering. SEM and AFM imaging show an homogeneous mineral distribution on the RCP matrix mineralized in presence of Mg, which provides a surface roughness similar to that found in bone. Preliminary in vitro tests

							with pre-osteoblast cell line show good cell-material interaction on the matrices prepared in the presence of Mg. To the best of our knowledge this work represents the first attempt to mineralize recombinant collagen type I derived protein proving the simultaneous effect of the organic phase (RCP) and Mg on ACP stabilization. This study opens the possibility to engineer, through biomineralization process, advanced hybrid matrices for bone regeneration.
7	Perivascular scaffolds loaded with adipose-derived stromal cells attenuate progression of abdominal aortic aneurysm in rats	Parvizi, Petersen, van Spreuwel-Goossens, Kluijtmans, Harmsen,	2017	J. Biomed Mater Res Part A 2018	UMCG, BMM	Available	Abdominal aortic aneurysm (AAA) is the pathological dilation and weakening of the abdominal aorta wall. Inflammation, degradation of the extracellular matrix (ECM) and loss of smooth muscle cells and skewing of their function are pivotal in AAA pathology. We developed a recombinant collagen based patch (RCP) to provide structural integrity and deliver Adipose tissue-derived Stromal Cells (ASC) for repair. Patches supported adhesion and function as well as proliferation of ASC. ASC-loaded RCPs or bare patches, applied around the aorta after AAA induction in rats, both maintained structural integrity of the aortic wall at time of explant (2w). However, wall thinning, accompanied by loss of elastin fibers and loss of medial SMC, was only attenuated in ASC-loaded RCP-treated AAA rats. Interestingly, this coincided with migration of ASC into the media and a reduced influx of macrophages. We hypothesize that the medially-migrated ASC dampened or skewed the adverse innate immunity and thus suppressed SMC apoptosis, phenotypic skewing and elastin degradation. We conclude that the periadventitial delivery of ASC with RCP suppresses development and progression of AAA, which is has an expected future clinical benefit in combination with an appropriate early screening program of patients at risk for aneurysms
8	Foreign body reaction against ADSC loaded RCP microspheres	Parvizi, Plantinga, van Spreuwel-Goossens, van Dongen, Kluijtmans, Harmsen	2016	Available as thesis chapter. To be resubmitted	UMCG, BMM	To be resubmitted	
9	Collagen I derived recombinant protein microspheres as novel delivery vehicles for bone morphogenetic protein-2	Mumcuoglu, de Miguel, Jekhmane, Nickel, van Leeuwen, van Osch, Kluijtmans	2017	Mat. Science and Engineering, part C, 84, (2018), 271-280	BioInspire	Available	Bone morphogenetic protein-2 (BMP-2) is a powerful osteoinductive protein; however, there is a need for the development of a safe and efficient BMP-2 release system for bone regeneration therapies. Recombinant extracellular matrix proteins are promising next generation biomaterials since the proteins are well-defined, reproducible and can be tailored for specific applications. In this study, we have developed a novel and versatile BMP-2 delivery system using microspheres from a recombinant proteinbased on human collagen I (RCP). In general, a two-phase release pattern was observed while the majority of BMP-2 was retained in the microspheres for at least two weeks. Among different parameters studied, the crosslinking and the size of the RCP microspheres changed the in vitro BMP-2 release kinetics significantly. Increasing the chemical crosslinking (hexamethylene diisocyanide) degree decreased the amount of initial burst release (24 h) from 23% to 17%. Crosslinking by dehydrothermal treatment further decreased the burst release to 11%. Interestingly, the 50 and 72 µm-sized spheres showed a significant decrease in the burst release compared to 207-µm sized spheres. Very importantly, using a reporter cell line, the released BMP-2 was shown to be bioactive. SPR data showed that N-terminal sequence of BMP-2 was important for the binding and retention of BMP-2 and suggested the presence of a specific binding epitope on RCP (KD: 1.2 nM). This study demonstrated that the presented RCP microspheres are promising versatile BMP-2 delivery vehicles.
10	Ice-Templating of Anisotropic Structures with High Permeability	Pawelec, van Boxtel, Kluijtmans	2017	Mat. Science and Engineering, part C, 17, 2017, 628-636	BioInspire	Available	Nutrient diffusion and cellular infiltration are important factors for tissue engineering scaffolds..Maximizing both, by optimizing permeability and scaffold architecture, is important to achieve functional recovery. The relationship between scaffold permeability and structure was explored in anisotropic scaffolds from a human collagen I based recombinant peptide (RCP). Using ice-templating, scaffold pore size was controlled (80–600 µm) via the freezing protocol and solution composition. The transverse pore size, at each location in the scaffold, was related to the freezing front velocity, via a power law, independent of the freezing protocol. Additives which interact with ice growth, in this case 1wt% ethanol, altered ice crystallization and increased the pore size. Variations in composition which did not affect the freezing, such as 40 wt% hydroxyapatite (HA), did not change the scaffold structure, demonstrating the versatility of the technique. By controlling the pore size, scaffold permeability could be tuned from 0.17 × 10−8 to 7.1 × 10−8 m2, parallel to the aligned pores; this is several orders of magnitude greater than literature

							values for isotropic scaffolds: 10−9–10−12m2. In addition, permeability was shown to affect the migration of osteoblast-like cells, suggesting that by making permeability a design parameter, tissue engineering scaffolds can promote better tissue integration.
11	Biomineralization of Recombinant Peptide Scaffolds: Interplay among Chemistry, Architecture, and Mechanics	K. M. Pawelec, Sebastiaan G.J.M. Kluijtmans	2017	ACS Biomater. Sci. Eng., 2017, 3, 1100–1108	BioInspire	Available	Biomineralized scaffolds are an attractive option for bone tissue engineering, being similar to native bone. However, optimization is difficult, due to the complex interplay among architecture, chemistry, and mechanics. Utilizing biomimetic nucleation, linear mineralized scaffolds were created from a collagen type I based recombinant peptide (RCP). Osteoblast mineralization was assessed, in response to changes in scaffold architecture, hydroxyapatite (HA) content, and mechanics. Changes in scaffold pore size (150−450 μm) had little effect on mRNA levels but influenced cell proliferation, achieving a balance between nutrient diffusion and surface area for cell attachment at 300 μm. Increasing the scaffold mechanical strength, from 2.9 to 5.2 kPa, enhanced the expression of osteocalcin, a late marker of mineralization. Further addition of HA, up to 20 wt %, increased osteoblast mineralization, without altering the compressive modulus. Thus, it was shown that architectural cues influence cellular proliferation, while the scaffold chemistry and mechanics independently contribute to gene expression.
12	Osteogenesis and Mineralization of Mesenchymal Stem Cells in Collagen Type I Based Recombinant Peptide Scaffolds	Pawelec, Kendell; Confalonieri, Davide; Ehlicke, Franziska; van Boxtel, Huibert Walles, Heike; Kluijtmans, Bas	2017	J Biomed Mater Res A. 2017, 105, 1856-1866	BioInspire	Available	Recombinant peptides have the power to harness the inherent biocompatibility of natural macromolecules, while maintaining a defined chemistry for use in tissue engineering. Creating scaffolds from peptides requires stabilization via crosslinking, a process known to alter both mechanics and density of adhesion ligands. The chemistry and mechanics of linear scaffolds from a recombinant peptide based on human collagen type I (RCP) was investigated after crosslinking. Three treatments were compared: dehydrothermal treatment (DHT), hexamethylene diisocyanate (HMDIC), and genipin. With crosslinking, mechanical properties were not significantly altered, ranging from 1.9 to 2.7 kPa. However, the chemistry of the scaffolds was changed, affecting properties such as water uptake, and initial adhesion of human mesenchymal stem cells (hMSCs). Genipin crosslinking supported the lowest adhesion, especially during osteoblastic differentiation. While significantly altered, RCP scaffold chemistry did not affect osteoblastic differentiation of hMSCs. After four weeks in vitro, all scaffolds showed excellent cellular infiltration, with up-regulated osteogenic markers (RUNX2, Osteocalcin, Collagen type I) and mineralization, regardless of the crosslinker. Thus, it appears that, without significant changes to mechanical properties, crosslinking chemistry did not regulate hMSC differentiation on scaffolds from recombinant peptides, a growing class of materials with the ability to expand the horizons of regenerative medicine
13	A Novel Injectable Recombinant Collagen I Peptide – based Macroporous Microcarrier Allows Superior Expansion of C2C12 and Human Bone Marrow - derived Mesenchymal Stromal Cells and Supports Deposition of Mineralized Matrix	Davide Confalonieri, Margherita La Marca, Elisabeth Marianna Wilhelmina Maria van Dongen, Heike Walles, Franziska Ehlicke	2017	Tissue Engineering Part A, 23, 2017, 946-957	BioInspire	Available	The development of scaffold formulations based on extracellular matrix (ECM)-inspired synthetic materials constitutes an important resource for the advance of cell-based therapies in bone tissue engineering approaches, where both cell and scaffold implantation are often needed. Culturing cells on porous microcarriers (MCs) allows cell expansion in a three-dimensional microenvironment and constitutes a possible solution for minimally invasive cell and scaffold simultaneous delivery, but the reduced pore dimension and pore interconnection diameter of several commercially available MCs limits de facto cell ingrowth, and ultimately their suitability for in vivo cell delivery. In this study we investigated the potential of a new macroporous MC based on a collagen I-based recombinant peptide (Cellnest™) for C2C12 cells and human bone marrow-derived mesenchymal stromal cells (hBMSCs) expansion and we analyzed the influence of dehydrothermal (DHT), hexamethylene diisocyanate (HMDIC), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) cross-linking strategies on cell vitality, proliferation, and hBMSCs differentiation. We established a double emulsification protocol for the manufacturing of MCs characterized by external pores of 20-40 μm diameter, 73% porosity, and 20 ± 3 μm pore interconnection diameter supporting cell ingrowth and proliferation into the MC. MCs cross-linked with DHT and HMDIC supported higher cell proliferation comparing to a commercially available equivalent over the course of 7 days and resulted in higher cell yield by day 28. Moreover, while hBMSCs expansion on Cellnest-MCs did not lead to a significant upregulation of the early markers of osteogenic differentiation Col1a1 and Runx2, their differentiation potential into the osteogenic lineage was preserved when cultured in differentiation medium, as confirmed by mineralized ECM deposition. We believe that Cellnest-MCs will help in reaching clinically relevant cell quantities and ultimately help in accelerating the translation of cell-based therapies for bone tissue engineering in the clinical practice.

14	New bioactive bone-like microspheres with intrinsic magnetic properties obtained by bio-inspired mineralisation process	Tatiana Marisa Fernandes Patrício, Silvia Panseri, Monica Sandri, Anna Tampieri, Simone Sprio	2017	Mat. Science and Engineering, part C, 17, 2017, 613-623	BioInspire	Available	A bio-inspired mineralisation process was investigated and applied to develop novel hybrid magnetic materials by heterogeneous nucleation of Fe2+/Fe3+-doped hydroxyapatite nanocrystals onto a biopolymeric matrix made of a Type I collagen-based recombinant peptide (RCP). The effect of the synthesis temperature on the phase composition, crystallinity and magnetic properties of the nucleated inorganic phase was studied. The as-obtained magnetic materials were then engineered, by using a water-in-oil emulsification process, into hybrid magnetic microspheres, which were stabilized by de-hydrothermal treatment yielding cross-linking of the macromolecular matrix. Thorough investigation of the physicochemical, morphological and biological properties of the new hybrid microspheres, as induced by the presence of the inorganic nanophase and controlled iron substitution into hydroxyapatite lattice, revealed bone-like composition, good cytocompatibility, designed shape and size, and tailored magnetization. Such features are interesting and promising for application as new biomaterials with ability of remote activation and control by using external magnetic fields, for smart and personalized applications in medicine, particularly in bone tissue regeneration.
15	Biomineralized Recombinant Collagen-Based Scaffold Mimicking Native Bone Enhances Mesenchymal Stem Cell Interaction and Differentiation	Gloria Belén Ramírez-Rodrígueza, Monica Montesia, Silvia Panseri, Monica Sandri, Simone Sprio and Anna Tampieri	2017	Tissue Engineering Part A, 2017, 23(23-24), 1423-1435. https://doi.org/10.1089/ten.tea.2017.0028	BioInspire	Available	The need of synthetic bone grafts that recreate from macro- to nanoscale level the biochemical and biophysical cues of bone extracellular matrix has been a major driving force for the development of new generation of biomaterials. In this study, synthetic bone substitutes have been synthesized via biomimetic mineralization of a recombinant collagen type I-derived peptide (RCP), enriched in tri-amino acid sequence arginine-glycine-aspartate (RGD). Three-dimensional (3D) isotropic porous scaffolds of three different compositions are developed by freeze-drying: non-mineralized (RCP, as a control), mineralized (Ap/RCP), and mineralized scaffolds in the presence of magnesium (MgAp/RCP) that closely imitate bone composition. The effect of mineral phase on scaffold pore size, porosity, and permeability, as well as on their in vitro kinetic degradation, is evaluated. The ultimate goal is to investigate how chemical (i.e., surface chemistry and ion release from scaffold) together with physical signals (i.e., surface nanotopography) conferred via biomimetic mineralization can persuade and guide mesenchymal stem cell (MSC) interaction and fate. The three scaffold compositions showed optimum pore size and porosity for osteoconduction, without significant differences between them. The degradation tests confirmed that MgAp/RCP scaffolds presented higher reactivity under physiological condition compared to Ap/RCP ones. The in vitro study revealed an enhanced cell growth and proliferation on MgAp/RCP scaffolds at day 7, 14, and 21. Furthermore, MgAp/RCP scaffolds potentially promoted cell migration through the inner areas reaching the bottom of the scaffold after 14 days. MSCs cultured on MgAp/RCP scaffolds displayed higher gene and protein expressions of osteogenic markers when comparing them with the results of those MSCs grown on RCP or Ap/RCP scaffolds. This work highlights that mineralization of recombinant collagen mimicking bone mineral composition and morphology is a versatile approach to design smart scaffold interface in a 3D model guiding MSC fate
16	Recombinant human collagen-based microspheres mitigate cardiac conduction slowing induced by adipose tissue-derived stromal cells	Nicoline Smit et al	2017	PLOS One, August 24, 2017 https://doi.org/10.1371/journal.pone.0183481	BMM	Available (open access)	Stem cell therapy to improve cardiac function after myocardial infarction is hampered by poor cell retention, while it may also increase the risk of arrhythmias by providing an arrhythmogenic substrate. We previously showed that porcine adipose tissue-derived-stromal cells (pASC) induce conduction slowing through paracrine actions, whereas rat ASC (rASC) and human ASC (hASC) induce conduction slowing by direct coupling. We postulate that biomaterial microspheres mitigate the conduction slowing influence of pASC by interacting with paracrine signaling. It is the aim to investigate the modulation of ASC-loaded recombinant human collagen-based microspheres, on the electrophysiological behavior of neonatal rat ventricular myocytes (NRVM). Conclusion: The application of recombinant human collagen-based microspheres mitigates indirect paracrine conduction slowing through interference with a secondary autocrine myocardial factor.
17	Novel in situ gelling hydrogels loaded with recombinant collagen peptide microspheres as a slow release system induce ectopic bone formation	S. Fahmy-Garcia, D. Mumcuoglu, L. de Miguel, V. D. Dieleman, J. Witte-Bouma, B.C.J van der Eerden, M. van Driel , D. Eglin, J. A. N. Verhaar, S.G.J.M.	2018	Advanced Healthcare materials, 10.1002/adhm.201800507	BioInspire	Available	New solutions for large bone defect repair are needed. Here, in situ gelling slow release systems for bone induction are assessed. Collagen‐I based Recombinant Peptide (RCP) microspheres (MSs) are produced and used as a carrier for bone morphogenetic protein 2 (BMP‐2). The RCP‐MSs are dispersed in three hydrogels: high mannuronate (SLM) alginate, high guluronate (SLG) alginate, and thermoresponsive hyaluronan derivative (HApN). HApN+RCP‐MS forms a gel structure at 32 °C or above, while SLM+RCP‐MS and SLG+RCP‐MS respond to

		Kluijtmans, G.J.V.M van Osch, and E. Farrell					shear stress displaying thixotropic behavior. Alginate formulations show sustained release of BMP‐2, while there is minimal release from HApN. These formulations are injected subcutaneously in rats. SLM+RCP‐MS and SLG+RCP‐MS loaded with BMP‐2 induce ectopic bone formation as revealed by X‐ray tomography and histology, whereas HApN+RCP‐MS do not. Vascularization occurs within all the formulations studied and is significantly higher in SLG+MS and HApN+RCP‐MS than in SLM+RCP‐MS. Inflammation (based on macrophage subset staining) decreases over time in both alginate groups, but increases in the HApN+RCP‐MS condition. It is shown that a balance between inflammatory cell infiltration, BMP‐2 release, and vascularization, achieved in the SLG+RCP‐MS alginate condition, is optimal for the induction of de novo bone formation.
18	Introduction to a new cell transplantation platform via recombinant peptide petaloid pieces and its application to islet transplantation with mesenchymal stem cells	Kentaro Nakamura, Reiko Iwazawa, Yasuhiro Yoshioka	2016	Transplant International, Volume 29, Issue 9, September 2016, Pages 1039–1050	RMRL	Available (open access)	Cell death cluster in transplanted cells remains a critical obstacle for regeneration strategies. This study describes a novel platform for cell transplantation (CellSaic) consisting of human mesenchymal stem cells (hMSCs) and petaloid pieces of recombinant peptide (RCP), which can prevent cell death by arranging the cells in a mosaic. When hMSC CellSaics were subcutaneously implanted into NOD/SCID mice, hMSC CellSaics prevented cell death and accelerated angiogenesis in the graft, compared to the findings obtained on solely implanting cell spheroids. Additionally, we examined the application of CellSaic for subcutaneous cotransplantation of 200 rat islets with 2 9 105 hMSCs into diabetic mice. As the results of blood glucose levels at 1 M, the islet-only group was 398 30 mg/dl and the islets with hMSCs group were 180 65 mg/dl. On the other hand, the islets with hMSCs CellSaic group showed 129 15 mg/dl and significantly improved glucose tolerance ($P < 0.05$). Additionally, we showed that the surface texture of the RCP petaloid pieces played an important role in graft survival and angiogenesis. It is anticipated that CellSaic will be used as a new platform for cell transplantation and tissue regeneration.
19	Recombinant collagen I peptide microcarriers for cell expansion and their potential use as cell delivery system in a bioreactor model	Melva Suarez, Davide Confalonieri, Suzan van Dongen, Heike Walles	2017	Journal of Visualized Experiments	BioInspire	Available	Tissue engineering is a promising field, focused on developing solutions for the increasing demand on tissues and organs regarding transplantation purposes. The process to generate such tissues is complex, and includes an appropriate combination of specific cell types, scaffolds and physical or biochemical stimuli to guide cell growth and differentiation. Microcarriers represent an appealing tool to expand cells In a three-dimensional (3D) microenvironment, since they provide higher surface-to volume ratios and mimic more closely the in vivo situation compared to traditional two-dimensional methods. The vascular system, supplying oxygen and nutrients to the cells and ensuring waste removal, constitutes an important building block when generating engineered tissues. In fact, most constructs fail after being implanted due to lacking vascular support. In this study, we present a protocol for endothelial cell expansion on recombinant collagen-based microcarriers under dynamic conditions in spinner flask and bioreactors, and we explain how to determine in this setting cell viability and functionality. In addition, we propose a method for cell delivery for vascularization purposes, and a strategy to evaluate the cell vascularization potential in a perfusion bioreactor on a biological matrix. We believe that the use of the presented methods could lead to the development of new cell-based therapies for a large range of tissue engineering applications in the clinical practice.
20	Enhancement of cellular adhesion and proliferation by instillation of type I collagen base recombinant peptide solution into culture medium	Muraya, Kawasaki, Maekawa, Yoshioka, Akutsu	2018	Bioresearch open access	RMRL	submitted	
21	Effect of Superparamagnetic bone-like microspheres on osteoblast behaviour	Tatiana M. Fernandes Patrício, Silvia Panseri, Monica Montesi , Michele Iafisco, Monica Sandri, Anna Tampieri,	2018	Materials Science and Engineering: C Volume 96, March 2019, Pages 234-247 https://doi.org/10.1016/j.msec.2018.11.014	BioInspire	Requested	A bio-inspired mineralization process was applied to a macromolecular collagen type I-like peptide matrix (RCP) to obtain heterogeneous nucleation of hydroxyapatite phase presenting Fe2+/Fe3+ doping and the formation of hybrid slurries. Surface functionalization with citrate ions was a key process to improve the microspheres dispersion in osteogenic differentiation medium and to investigate the stability of the microspheres up to 28 days in physiological and inflammatory-mimicking

		Simone Sprio					conditions. Quantitative and qualitative analysis of phenotypic and genotypic expression of cells differentiation in the presence of the hybrid magnetic microspheres was carried out with MC3T3-E1 cell line and reported on good cell viability and a significant up-regulation of osteogenic markers (BGLAP, COL I and SPARC). The presented results open new perspectives in bone tissue applications by the use of these novel biomimetic magnetic microspheres, as magnetically active bone substitute with potential ability of drug carrier.
22	Injectable BMP-2 delivery system based on collagen-based microspheres and alginate induces bone formation in a time and dose dependent manner	D. Mumcuoglu, S. Fahmy-Garcia, R.Y. Ridwan, J. Nickel, E. Farrell, S.G.J.M. Kluijtmans, G.J.V.M van Osch	2018	2018 Volume No 35 – pages 242-254 DOI:10.22203/eCM	BioInspire	Available, open access	The aim of the current study was to reduce the clinically used supra-physiological dose of bone morphogenetic protein-2 (BMP-2) (usually 1.5 mg/mL), which carries the risk of adverse events, by using a more effective release system. A slow release system, based on an injectable hydrogel composed of BMP-2-loaded recombinant collagen-based microspheres and alginate, was previously developed. Time- and dose-dependent subcutaneous ectopic bone formation within this system and bone regeneration capacity in a calvarial defect model were investigated. BMP-2 doses of 10 µg, 3 µg and 1 µg per implant (50 µg/mL, 15 µg/mL and 5 µg/mL, respectively) successfully induced ectopic bone formation subcutaneously in rats in a time- and dose-dependent manner, as shown by micro-computed tomography (µCT) and histology. In addition, the spatio-temporal control of BMP-2 retention was shown for 4 weeks in vivo by imaging of fluorescently labelled BMP-2. In the subcritical calvarial defect model, µCT revealed a higher bone volume for the 2 µg of BMP-2 per implant condition (50 µg/mL) as compared to the lower dose used (0.2 µg per implant, 5 µg/mL). Complete defect bridging was obtained with 50 µg/mL BMP-2 after 8 weeks. The BMP-2 concentration of 5 µg/mL was not sufficient to heal a calvarial defect faster than the empty defect or biomaterial control without BMP-2. Overall, this injectable BMP-2 delivery system showed promising results with 50 µg/mL BMP-2 in both the ectopic and calvarial rat defect models, underling the potential of this composite hydrogel for bone regeneration therapies
23	in vitro model of osteonecrosis and evaluation of osteogenic potential of pastes	M. Maglio	2018	Submitted to Stem Cell International	BioInspire	rejected	
24	Therapeutic effects of a recombinant human collagen peptide bioscaffold with human adipose-derived stem cells on impaired wound healing after radiotherapy.	Mashiko, Takada, Wu , Kanayama , Feng , Tashiro, Asah, Sunaga, Hoshi, Kurisaki, Takato, Yoshimura	2018	J Tissue Eng Regen Med. 2018 May;12(5):1186-1194 doi: 10.1002/term.2647	RMRL	requested	Chronic changes following radiotherapy include alterations in tissue-resident stem cells and vasculatures, which can lead to impaired wound healing. In this study, novel recombinant human collagen peptide (rhCP) scaffolds were evaluated as a biomaterial carrier for cellular regenerative therapy. Human adipose-derived stem cells (hASCs) were successfully cultured on rhCP scaffolds. By hASC culture on rhCP, microarray assay indicated that expression of genes related to cell proliferation and extracellular matrix production was upregulated. Pathway analyses revealed that signaling pathways related to inflammatory suppression and cell growth promotion were activated as well as signaling pathways consistent with some growth factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and transforming growth factor beta (TGF-β1), although gene expression of these growth factors was not upregulated. These findings suggest the rhCP scaffold showed similar biological actions to cytokines regulating cell growth and immunity. In subsequent impaired wound healing experiments using a locally irradiated (20 Gray) mouse, wound treatment with rhCP sponges combined with cultured hASCs and human umbilical vein endothelial cells accelerated wound closure compared to wounds treated with rhCP with hASCs alone, rhCP only, and control (dressing alone), with better healing observed according to this order. These results indicating the therapeutic value of rhCP scaffolds as a topical biomaterial dressing and a biocarrier of stem cells and vascular endothelial cells for regenerating therapies. The combination of rhCP and functional cells was suggested to be a potential tool for revitalizing stem cell-depleted conditions such as radiation tissue damage
25	Human Recombinant Peptide Sponge Enables Novel, Less Invasive Cell Therapy for Ischemic Stroke	Michiyuki Miyamoto, Kentaro Nakamura , Hideo Shichinohe , Tomohiro Yamauchi, Masaki Ito, Hisayasu Saito, Masahito Kawabori, Toshiya Osanai, Tasuku Sasaki,	2018	Stem Cells International Volume 2018, Article ID 4829534, 8 pages https://doi.org/10.1155/2018/4829534	RMRL	Available (open access)	Bone marrow stromal cell (BMSC) transplantation has the therapeutic potential for ischemic stroke. However, it is unclear which delivery routes would yield both safety and maximal therapeutic benefits. We assessed whether a novel recombinant peptide (RCP) sponge, that resembles human collagen, could act as a less invasive and beneficial scaffold in cell therapy for ischemic stroke. BMSCs from green fluorescent protein-transgenic rats were cultured and Sprague–Dawley rats were subjected to permanent middle cerebral artery occlusion (MCAo). A BMSC-RCP sponge construct was transplanted onto the ipsilateral intact neocortex 7 days after MCAo. A BMSC suspension or vehicle was transplanted into the ipsilateral striatum. Rat motor function was serially evaluated and histological analysis was

		Kiyohiro Houkin, and Satoshi Kuroda					performed 5 weeks after transplantation. The results showed that BMSCs could proliferate well in the RCP sponge and the BMSC-RCP sponge significantly promoted functional recovery, compared with the vehicle group. Histological analysis revealed that the RCP sponge provoked few inflammatory reactions in the host brain. Moreover, some BMSCs migrated to the peri-infarct area and differentiated into neurons in the BMSC-RCP sponge group. These findings suggest that the RCP sponge may be a promising candidate for animal protein-free scaffolds in cell therapy for ischemic stroke in humans.
26	Synthetic extracellular matrix structures composed of recombinant collagen peptides and strong supramolecular interactions	Sergio Spaans, Peter-Paul K. H. Fransen, Maaike J. G. Schotman, Ruben van der Wulp, René P. M. Lafleur, Sebastiaan G. J. M. Kluijtmans, Patricia Y. W. Dankers*	2019	Submitted to Biomacromolecules	TU/e	Accepted with minor revisions	The natural extracellular matrix is crucial for the maintenance of biological activity such as, e.g. adhesion, proliferation and survival of cells. In this work, natural matrices are mimicked via the development of synthetic hydrogels based on a 571-residue recombinant collagen peptide (RCP) which is crosslinked via supramolecular ureido-pyrimidinone (UPy) interactions. By grafting supramolecular UPy functionalities onto the backbone of RCP (UPy-RCP), increased control over the polymer structure, assembly, gelation and mechanical properties was achieved. Accordingly, by increasing the degree of UPy-functionalization on RCP, cardiomyocyte progenitor cells (CPCs) were cultured on “soft” (~26 kPa) versus “stiff” (~68-190 kPa) UPy-RCP hydrogels. Interestingly, increased stress fiber formation, focal adhesions, and proliferation were observed on stiffer compared to softer substrates. Furthermore, CPC mechano-responsive behaviour was elucidated through qualitative assessment of nuclear localisation of Yes-associated protein (YAP) on stiffer and softer substrates. With this study, a new class of natural ECM-mimicking and mechanically controllable synthetic hydrogel was developed, which could be used to create the ideal supporting matrix for cell-based tissue regeneration.
27	Photo-crosslinkable recombinant collagen mimics for tissue engineering applications	Liesbeth Tytgata,b, Marica Markovic, Taimoor H. Qazie, Maxime Vagenende, Fabrice Bray, José C. Martins, Christian Rolando, Hugo Thienpont, Heidi Ottevaere, Aleksandr Ovsianikov, Peter Dubruel, Sandra Van Vlierberghe	2019	Published in J. Materials Chemistry B http://dx.doi.org/10.1039/C8TB03308K	UGent	Available	Gelatin is frequently used in various biomedical applications. However, gelatin is generally extracted from an animal source which can result in issues with reproducibility as well as pathogen transmittance. Therefore, we have investigated the potential of a recombinant peptide based on collagen I (RCPhC1) for tissue engineering applications and more specifically for adipose tissue regeneration. In the current paper, RCPhC1 was functionalized with methacrylamide photocrosslinkable moieties to enable subsequent UV-induced crosslinking in the presence of a photo-initiator. The resulting biomaterial (RCPhC1-MA) was characterized by evaluating the crosslinking behavior, the mechanical properties, the gel fraction, the swelling properties and the biocompatibility. The obtained results were compared with the data obtained for methacrylamide-modified gelatin (Gel-MA). The results indicated that the properties of RCPhC1-MA networks are comparable with those of animal-derived Gel-MA. RCPhC1-MA is thus an attractive synthetic alternative for animalderived Gel-MA and is envisioned to be applicable for a wide range of tissue engineering purposes.
28	Extemporaneous preparation of injectable and enzymatically degradable three-dimensional cell culture matrices from an animal component-free recombinant protein based on human collagen type I	Hiroyuki Kamata*, Satoko Ashikari-Hada, Akihiko Azuma, and Ken-ichiro Hata	2019	Macromolecular Rapid Communications	BTDC	Submitted	Hydrogels are indispensable tools for modern clinical and biomedical research. Their injectable formulations, among others, are considered important to realize safe and effective minimally invasive therapy in the field of regenerative medicine. Injected hydrogels are expected to serve as temporary scaffolds for therapeutically effective cells, and degrade after having functioned. Animal-derived natural polymers are well studied as the primary component of hydrogels, owing to their naturally acquired cell-adhesive and biodegradable properties. Without special modification, however, they lack injectability. Besides, the use of such biological materials is accompanied by the potential risks of immunogenic reactions or unknown pathogen contamination, which motivates for researchers to explore ideal injectable hydrogels prepared from synthetic polymers. Despite extensive research activities, such state-of-the art technology remains inaccessible to non-specialists. In this article, the design of a new injectable hydrogel platform that can be extemporaneously prepared from commercially available animal component-free materials is described. Their solidification time can be adjusted by choosing proper buffer conditions, which provides the hydrogels with injectability. The resultant hydrogels are confirmed enzymatically degradable. Anchorage-dependent cells can be encapsulated and cultured in the hydrogels with conventional equipment. This platform is accessible even to non-specialists, and expected to accelerate future cell-related research activities.
29	Follistatin Effects in Migration, Vascularization, and Osteogenesis in	Shorouk Fahmy-Garcia1,2, Eric	2019	Front. Bioeng. Biotechnol. 7:38. doi: 10.3389/fbioe.2019.00038	BioInspire	Available (open	he use of biomaterials and signaling molecules to induce bone formation is a promising approach in the field of bone tissue engineering. Follistatin (FST) is a glycoprotein able to bind irreversibly to activin A, a protein that has been reported to

	vitro and Bone Repair in vivo	Farrell3*, Janneke Witte-Bouma3, Iris Robbesom-van den Berge2, Melva Suarez4, Didem Mumcuoglu1,5, Heike Walles4, Sebastiaan G. J. M. Kluijtmans5, Bram C. J. van der Eerden2, Gerjo J. V. M. van Osch1,6, Johannes P. T. M. van Leeuwen2 and Marjolein van Driel2				access)	inhibit bone formation. We investigated the effect of FST in critical processes for bone repair, such as cell recruitment, osteogenesis and vascularization, and ultimately its use for bone tissue engineering. In vitro, FST promoted mesenchymal stem cell (MSC) and endothelial cell (EC) migration as well as essential steps in the formation and expansion of the vasculature such as EC tube-formation and sprouting. FST did not enhance osteogenic differentiation of MSCs, but increased committed osteoblast mineralization. In vivo, FST was loaded in an in situ gelling formulation made by alginate and recombinant collagen-based peptide microspheres and implanted in a rat calvarial defect model. Two FST variants (FST288 and FST315) with major differences in their affinity to cell-surface proteoglycans, which may influence their effect upon in vivo bone repair, were tested. In vitro, most of the loaded FST315 was released over 4 weeks, contrary to FST288, which was mostly retained in the biomaterial. However, none of the FST variants improved in vivo bone healing compared to control. These results demonstrate that FST enhances crucial processes needed for bone repair. Further studies need to investigate the optimal FST carrier for bone regeneration.