

## **Kuros Biosurgery AG**

### **Kuros Synthetic Matrices for Protein Delivery and Tissue Repair**

Kuros has developed a novel synthetic technology platform which is broadly applicable to injectable controlled release of proteins, peptides and small molecules (“drugs”). In this platform, the drug is incorporated in synthetic biodegradable and biocompatible hydrogels which crosslink under gentle conditions in-situ.

The platform offers customizable release, crosslinking, and degradation parameters, and allows for cell ingrowth and remodeling of the matrix material to optimize tissue repair and generation or drug delivery.

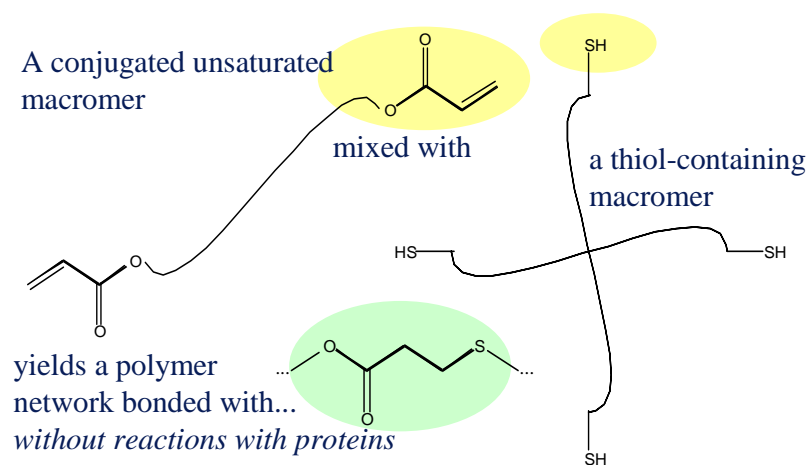
### **General Chemistry of Kuros Hydrogel Materials**

Kuros' synthetic hydrogel/matrix materials are formed from the reaction between a polymer containing multiple conjugated unsaturations, such as polyethylene glycol tetra-acrylate, with polymers that contain free thiols (see reaction scheme next page). The reaction between these thiol groups and the conjugated unsaturation is known as Michael addition reaction and has the following characteristics:

- (i) rapid gel formation in aqueous solution at physiologic conditions (pH, temperature). The reaction of the thiol onto the acrylate has high selectivity, i.e. the acrylate reaction with thiols is significantly faster than the reaction with amines or other groups found on proteins or peptides in the tissue environment;
- (ii) no by-products are formed by the reaction;
- (iii) no heat formation in the course of the reaction. The risk of damaging the surrounding tissue or the drug is therefore minimal;
- (iv) no external energy sources are needed to induce the reaction, which further minimizes the risk of damage of tissue or drug;
- (v) ease of preparation of the precursor molecules;
- (vi) wide variability of precursor molecules to design the release kinetic of the drug from the hydrogel

Kuros technology allows to use polyethylene glycol (PEG) as the principal component of the hydrogels, which enables the exploitation of the favourable properties of PEG with regard to drug delivery in the body, such as good biocompatibility (PEG is non-inflammatory), high hydrophilicity (no organic solvents), protein repellence, i.e. no hydrophobic regions as adsorption sites, low degradability by mammalian enzymes and ease of functionalization.

## Synthetic Gels with *Liquid-to-Solid Transitions*



D.L. Elbert, M. Lutolf, A.B. Pratt, S. Halstenberg

Kuros has two preferred formulations for its synthetic tissue repair matrices. The choice of matrix formulation depends on the application requirements. The hydrogel can be formulated to be either (a) *hydrolytically* or (b) *enzymatically degradable*.

### Hydrolytically degradable hydrogels

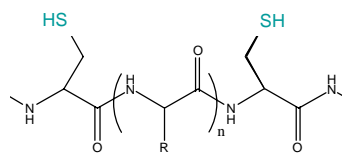
Through the reaction of the acrylate groups with the thiol groups of the precursor molecules a hydrogel with hydrolytically labile ester bonds are formed (see scheme above). The presence of these ester bonds allows the hydrogel to be degraded in the presence of water, a natural condition found *in vivo*. As the gel degrades, individual crosslinks are destroyed, allowing the gel to swell. Therefore, the extent of degradation can be measured by the swelling of the gel, with more advanced degradation represented by larger swelling ratios.

The gel formation kinetics and gel mechanical properties can be influenced by the cross-link density, concentration of PEG in the precursor solution, temperature and pH and part of the findings have been described in D.E. Elbert, A.B. Pratt, M.P.Lutolf, S.Halstenberg, J.A. Hubbell, J. of Controlled Release 76 (2001) 11-25 and D. Elbert, J. Hubbell, Biomacromolecules 2001,2,430-441.

### Enzymatically degradable hydrogels

By using a PEG precursor molecule end-functionalized with vinylsulfone groups ( $-\text{SO}_2-\text{CH}=\text{CH}_2$ ) (instead of acrylate groups) as the first precursor molecule and a linear peptide comprising two cysteine groups (the thiol group) as the second precursor molecule, a hydrogel is formed without any hydrolytically degradable ester bonds in the backbone. These hydrogels are only enzymatically degradable. The degradability is introduced by the amino acid sequence of the peptide precursor, which is cleavable by matrix metalloproteinases or plasmin. The rate of hydrolysis is controlled by changing the functionality of the precursor molecules as well as the sequence of the peptide precursor molecule. Apart from having enzymatically degradable sites as part of the amino acid sequence of the peptide precursor molecule, other functionalities can be introduced by designing the amino acid sequence of the precursor, like cell adhesion sites (RGD sites), growth factor binding sites, etc.

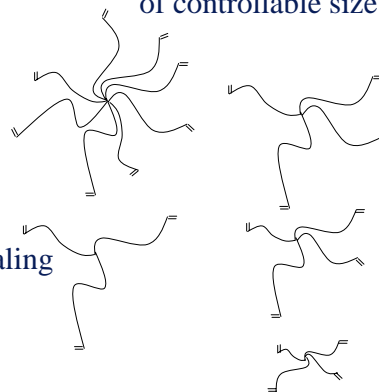
## Peptides add Functionality



Peptide in the middle could be:

- an adhesion site, for signaling
- a growth factor binding site, for signaling
- a protease substrate, for remodeling

Peptide with two cysteine residues, with > tri-functional PEG of controllable size



The procedure for delivery and in-situ formation of the hydrogel is simple:

A solution of PEG-thiol is mixed with the drug

The PEG-thiol/drug solution is mixed with the PEG-acrylate solution

Gelation is spontaneous, and occurs within 5-20 minutes depending on pH

Additional components (e.g. hydroxyapatite or calciumphosphates) can be added

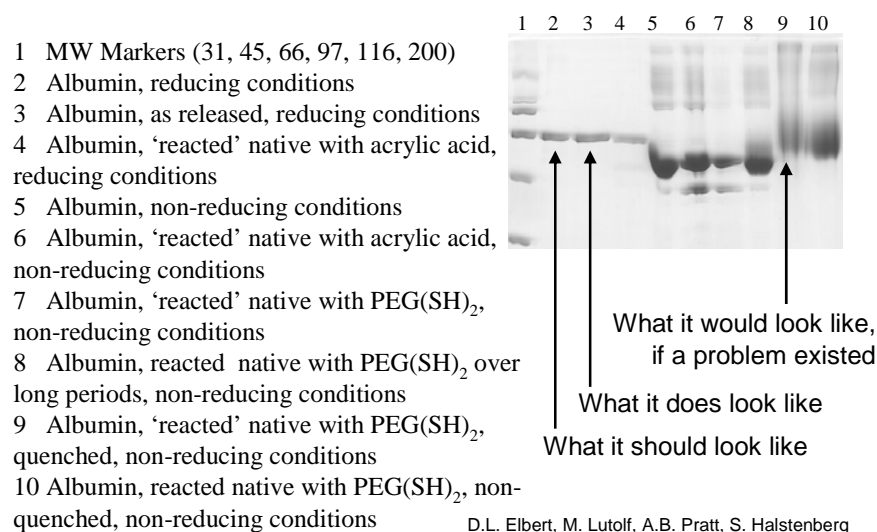
Mixing and concurrent application of the matrix to the body can be performed by using a dual compartment syringe. The data shown on the next pages is generally applicable to either formulation, particularly as relates to gelation control, protein incorporation, and gel degradation.

## Incorporation of Drugs

Virtually no drugs of interest have free cysteine groups on the protein surface which can interact with the gel formation. Thus, in case of hydrophobic drugs, the drug can be easily incorporated *without modification of the drug*.

If very hydrophilic and soluble drugs are used, the drug is modified with a cysteine or thiol such that it is selectively incorporated via covalent linkage to the hydrogel. No additional or non-intentional reaction of the drug with the matrix is observed due to the selectivity of the hydrogen formation.

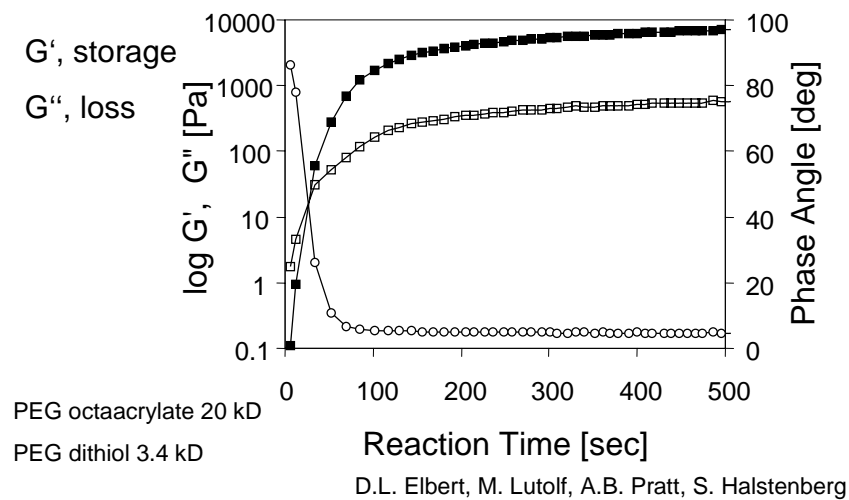
## Example with Albumin



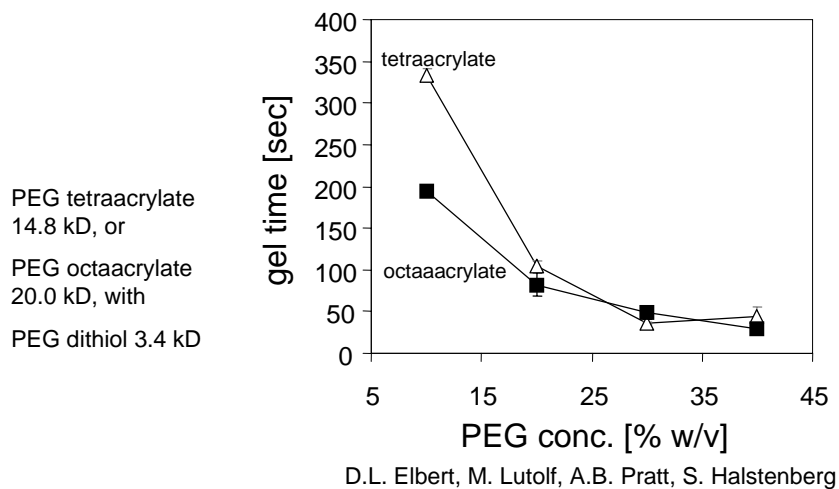
The data shown on the next pages is generally applicable to either formulation, particularly as it relates to gelation control, protein incorporation and gel degradation. Examples shown in hydrolytic gel if not indicated otherwise.

## Gelation time is controlled:

### Reaction Results in Gelation

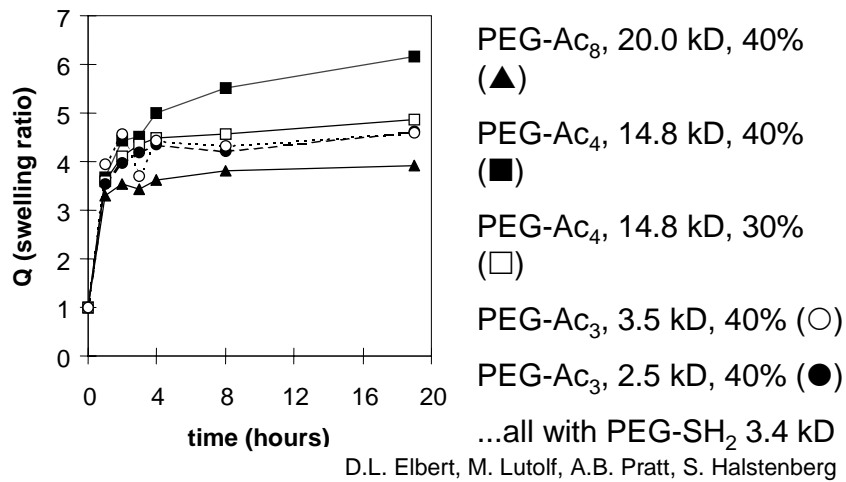


### Gel Times are Surgically Relevant

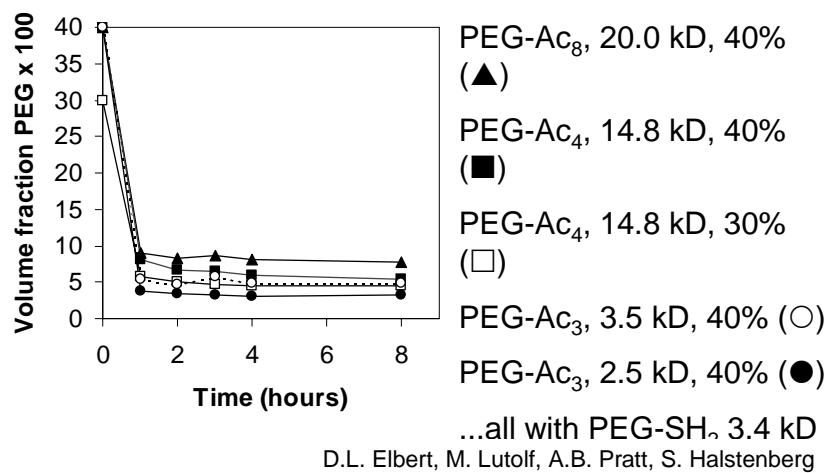


## Gel architecture can be controlled:

### Gel Swelling is a Function of Macromer Structure and Concentration



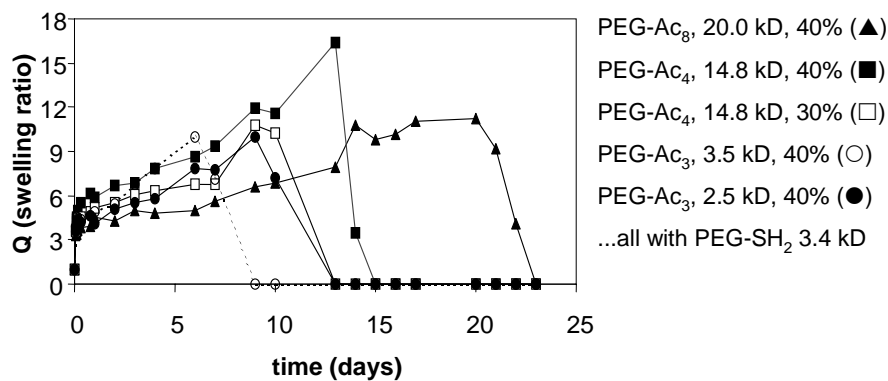
### Network Structure is a Function of Macromer Structure and Concentration



## Degradation can be controlled:

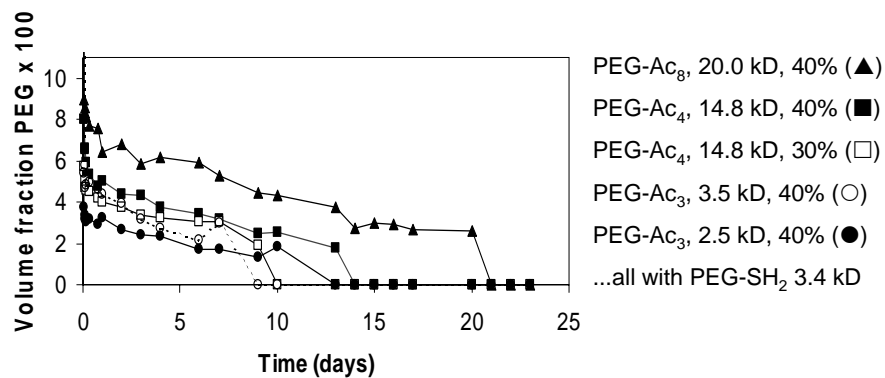
In case of hydrolytically degradable hydrogels, drug release is through the bulk degradation of the hydrogel rather than surface erosion. Degradation results in increased pore size and reduced PEG concentration, allowing solubilization and diffusion of the drug out of the hydrogel. Thus control of the degradation characteristics of the hydrogel is the driving factor for drug release.

## Degradation Can be Controlled



D.L. Elbert, M. Lutolf, A.B. Pratt, S. Halstenberg

## Degradation Can be Controlled

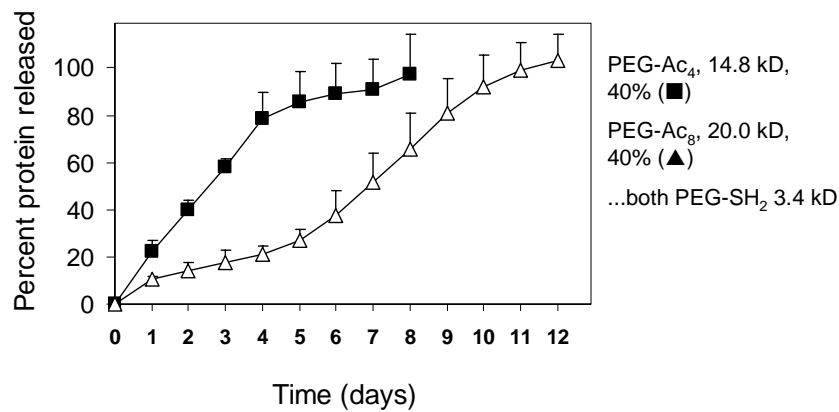


D.L. Elbert, M. Lutolf, A.B. Pratt, S. Halstenberg

## Drug release can be controlled

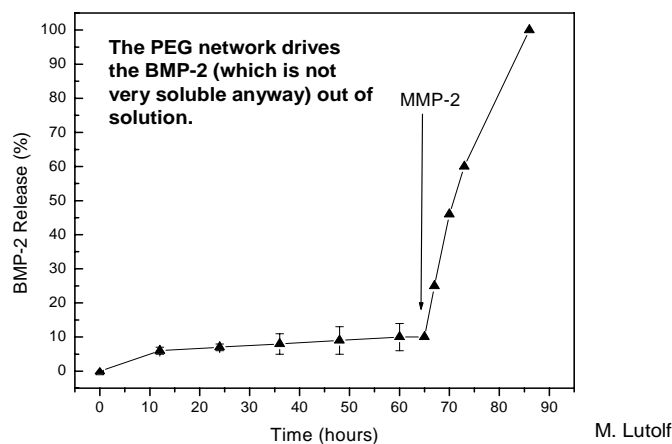
Drug release is dependent on gel architecture and degradation, as demonstrated in the figures below. In the second figure, the influence of the enzymatic degradation pathway is clearly demonstrated by the abrupt release of BMP from a Kuros PEG hydrogel upon exposure to a matrix metalloproteinase which degrades the peptide linkage in the gels.

### Protein is released dependent on gel architecture



D.L. Elbert, M. Lutolf, A.B. Pratt, S. Halstenberg

### BMP-2 can be incorporated physically and released by enzymatic gel degradation



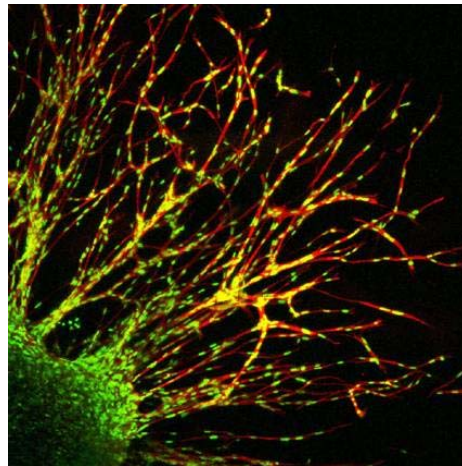
M. Lutolf

\* Example show above in enzymatic gel.

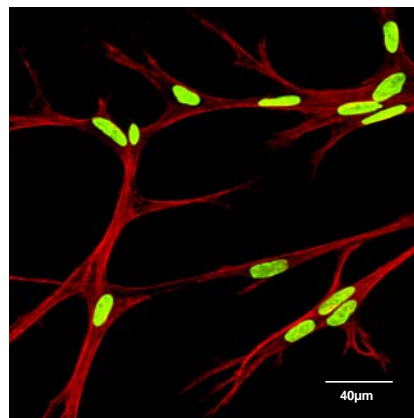
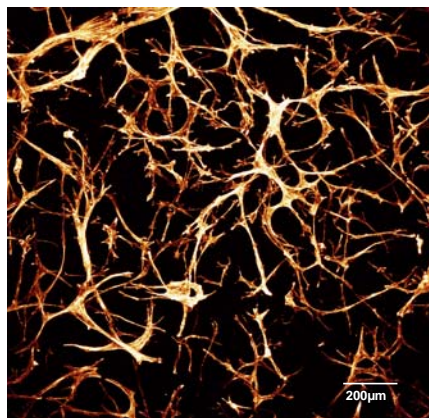
## Cells migrate and grow into the matrix in vitro:

In the examples below, we demonstrate that cells seeded into a Kuros PEG hydrogel migrate through the gel, locally degrading the gel such that the undegraded gel acts as a support for the newly formed network.

### Cell migration proceeds as a community

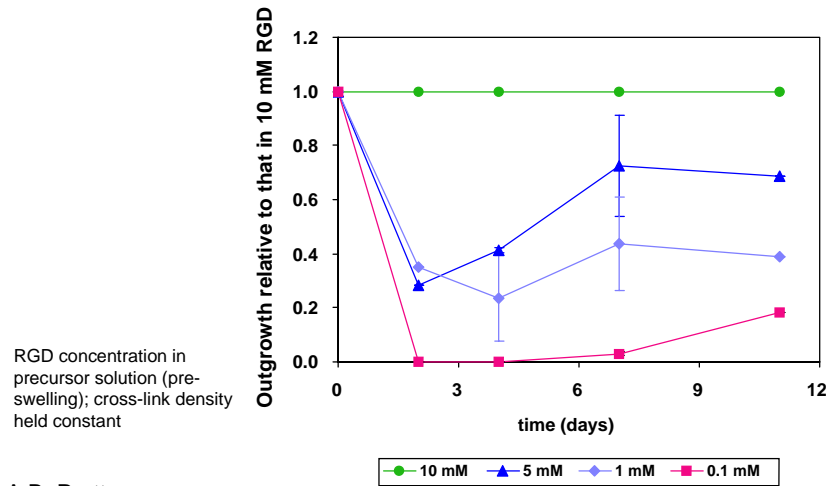


If seeded in 3D suspension, the cells form a 3D network eventually becoming “confluent”



\* Example show in enzymatic gels.

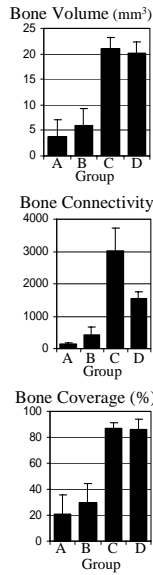
## Fibroblast migration can be changed by changing concentration of RGD ligand



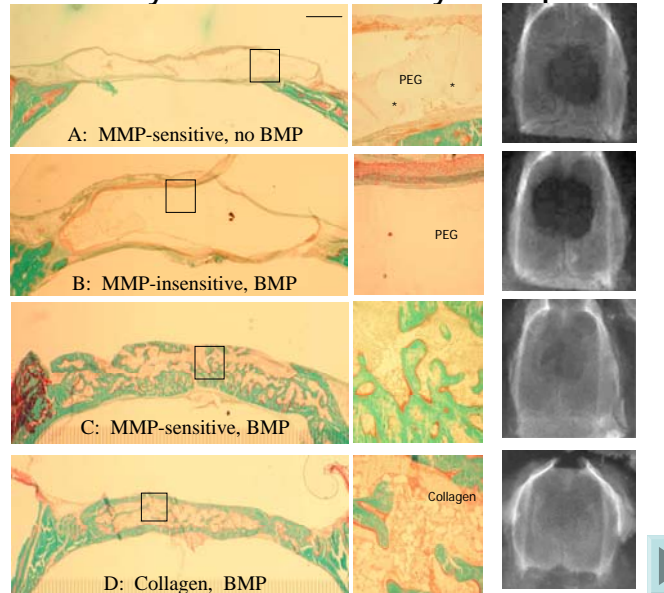
A.B. Pratt

Example show in enzymatic gels.

## Tissue regeneration by cell infiltration is seen in vivo



Bone formation *in vivo* corresponds to enzymatic sensitivity and protein

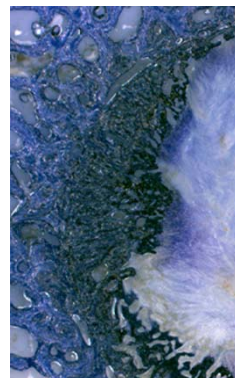


M. Lutolf, et al. Nat Biotechnol. 2003, 21:513-8

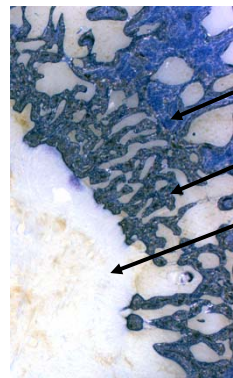
\* Example show in enzymatic gel.

## Cell infiltration and tissue formation in degradable gels with adhesion peptides is observed in sheep drill defects

Note that these gels contain no growth factor, and have not been optimized yet for cell ingrowth in this model



Sheep femur head drill defect #1



Sheep femur head drill defect #2

8mm drill defect at 8 weeks

## Competitive Comparison of Kuros Materials

Kuros intent is to deliver drugs, in particular proteins or peptides, in an injectable synthetic matrix for use in tissue repair and applications, in which the injectable matrix forms a monolith after injection around or into a device, defect or wound.

There are several known injectable depots. However, none offer the full scope of benefits of the Kuros technology

The key technical design criteria for this matrix are:

- No binding of the protein into the matrix chemically
- Controlled gelation in-situ
- Controlled release time
- Cell in-growth into the matrix for optimal tissue regeneration
- Ease of use optimized for surgeon and surgical suite usage
- Biocompatibility
- Retention of protein native conformation
- Ease of manufacturing and storage
- Patent protection

In the following pages please find:

- A comparison table between Kuros injectable matrix and other injectable depots
- An overview of the Kuros technology and data corresponding to the design criteria

	No covalent or strong matrix interaction?	Controlled gelation times?	Control of release times and profiles?	Cell in-growth for tissue repair?	Biocompatible?	Tertiary structure stability likely?	Easy use and handling?	Easy material storage and manufacturing?
KUROS Matrix	<b>Yes</b> Selectivity of thiol and acrylate, combined with rare free cysteine, results in v low protein modification	<b>Yes</b> Based on catalyst concentration	<b>Yes</b> Based on crosslink density, solubility, and degradation characteristics	<b>Yes</b> Addition of RGD and other peptides controls rate of cell infiltration	<b>Yes</b>	<b>Yes</b> Hydrogel	<b>Yes</b> Simple pre-mixing of materials or use of static mixer for "on demand" delivery	<b>Yes</b> Lyophilized or frozen
PEG hydrogel Amine-NHS	<b>No</b> Reactivity of NHS with amines on protein can be high	Yes Based on catalyst concentration	Yes Based on crosslink density, solubility, and degradation characteristics		Yes	Yes Hydrogel	Yes Simple pre-mixing of materials or use of static mixer for "on demand" delivery	
PEG hydrogel Photopolymerized	<b>No</b> Reactivity of photopolymerization of acrylate with amine on protein can be observed	Yes Based on light intensity and photoactivator concentration	Yes Based on crosslink density, solubility, and degradation characteristics		Yes	Yes Hydrogel	<b>No</b> External instrumentation required, along with fiberoptic system for polymerization	
Reverse phase or thermo-responsive hydrogels	Yes	<b>Low</b> Notoriously difficult to control for insitu gelation	<b>Low</b> Limited capacity to change polymeric degradation times or implant crosslink structure	<b>Low</b> Based on polymers with no known cell adhesion mechanism	Yes	Yes Hydrogel	<b>No</b> But dependent on need for controlled gelation time	
Inorganic (e.g. TCP)	<b>No</b> Retention is via adsorption of the protein onto the surface of the inorganic	<b>Low</b>	<b>Low</b> Based on dissolution of the inorganic or desorption of the protein from the particle surface	Somewhat Control through degradation of the TCP.	Yes	<b>Unknown</b> Surface adsorption can result in change to tertiary structure and thus functionality	<b>Limited</b> Very liquid initial properties followed by very rapid onset of setting, similar to PMMA	Yes
Unmodified Fibrin Sealant	Yes	Yes Set by thrombin concentration.	<b>Low</b> Based on solubility of the protein in the matrix	Yes	Yes Generally considered biocompatible	<b>Unknown</b> Unknown interaction between fibrin protein and protein of interest	Yes Delivery devices already optimized for surgeon handling and used in orthopaedic surgery	Yes Frozen or lyophilized forms available

## **Kuros injectable synthetic matrices are feasible for product development**

- Matrices have desired characteristics of handling, biocompatibility, cell ingrowth, protein incorporation and release, etc.
- GMP manufacturing for PEG matrix components is in place with large chemical synthesis company (including validated process, analytics, etc)
- Materials can be stored lyophilized
- Shelf life is >2 years at 4C
- Materials costs are <5-10 EUR per 10ml application for polymer; peptides (if used) add additional cost.
- Additives can be included (eg. TCP)
- Delivery device is simple 2 barrel syringe or related applicator
- Patent life for Kuros matrices is long (>2015)