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Supramolecular Biomaterials

Introducing a Modular Approach

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The increasing incidence of organ failure in the Western world, mainly caused by ageing of the population, is responsible for rising healthcare expenses. The number of patients requiring organ replacement therapy is expected to steadily increase in the future. At present, patients with organ failure rely on organ transplantation or costly treatments that help correct the systemic effects of organ malfunction. However, the poor availability of donor organs, and the apparent side-effects of conventional organ replacement therapies, has driven the quest for alternative and durable organ replacement solutions. For various tissues the development of bioartificial organs, i.e. hybrid constructs containing cells and biomaterials, is currently explored. It is expected that these bioartificial organs have far-reaching implications in improving life expectancy and quality of life.

Initially, biomaterials were used as inert prostheses to restore malfunctioning of organs in the human body, of which hip implants and spinal cord intervertebral disks are clear examples. However, difficulties arise when soft tissues and organs, as heart valves and kidney have to be transplanted. In these cases inert implants cannot be used. Therefore, regenerative medicine, in which devices specifically interact with the human body to achieve local regeneration, is a promising strategy to tackle this problem.

Tissue engineering

Biomaterials are extensively studied for their use in regenerative medicine, as drug delivery devices or as scaffolds for tissue engineering. The tissue engineering concept implies the formation of a new tissue by means of culturing of the patient's own cells on a polymer scaffold (fig. 1). For correct tissue formation, the cells have to receive the right signals to form the tissue, while the polymer scaffold has to be degraded. In time, the newly formed tissue has to have ample mechanical strength to replace the scaffold. Therefore, ideal scaffolds consist of biomaterials which are, next to being biocompatible and processable, able to fulfil the biomaterials trinity of tunable mechanical properties, biodegradability and bioactivity. Ultimately, the engineered tissue has to be transplanted into the patient. In this way, custom-made implants are produced.

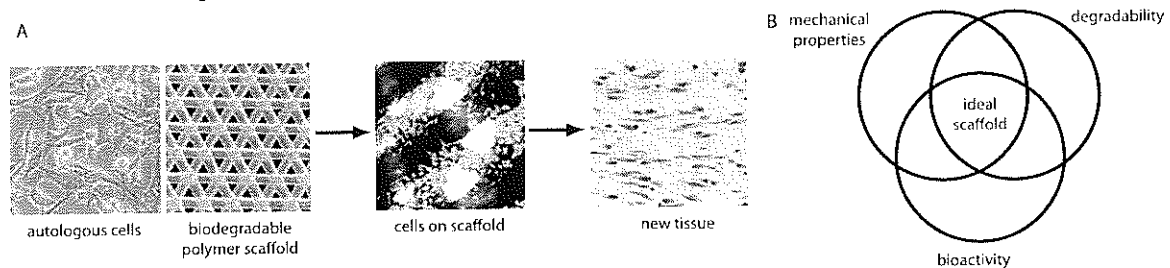


Figure 1. A. The tissue engineering concept and B. the biomaterials trinity.

A supramolecular and modular approach

Current approaches use conventional polymeric materials as scaffold. In this PhD project we show an innovation in polymer material design. A new biomaterials concept is introduced by means of directed non-covalent interactions. The advantages of this so-called supramolecular approach enclose benefits in processability, mechanical tunability and most importantly biofunctionalization.

Hydrogen bond formation is an example of a highly directed and specific non-covalent interaction. Therefore, a self-complementary quadruple hydrogen-bonding 2-ureido-4[1H]-pyrimidinone (UPy) moiety has been developed to be used as building block for supramolecular aggregates and polymers. This UPy-unit, which strongly dimerizes in organic solvents and takes care of chain extension of

relatively short end-functionalized UPy-prepolymers in the bulk ($K_a = 10^6\text{-}10^7 \text{ L} \cdot \text{mol}^{-1}$), was used for the construction of supramolecular biomaterials. The reversible nature of these hydrogen bonding interactions (with life times between 0.1-1 sec) creates responsive materials and allows for a modular approach. These new materials show mechanical properties similar to conventional polymers, without losing their reversible nature. The objective of the research was to produce both passive and active scaffolds by introducing a supramolecular and modular approach using these UPy-UPy interactions (fig. 2). The creation of a toolbox with different UPy-modified polymers, bioactive molecules and imaging probes allows for the off-the-shelf assembly of biomaterials by easy mixing without the need for additional elaborated synthesis steps. Furthermore, this supramolecular concept bridges the gap between simply (non-covalent) mixing and covalent modification of polymers with bioactive molecules and is therefore very promising. A modular approach to passive scaffolds was used by intimate mixing of UPy-modified prepolymers to tune the mechanical properties and biodegradability of the biomaterials. Active scaffolds were made by incorporation of UPy-modified bioactive compounds such as bioactive peptides and proteins using the same mix-and-match principle.

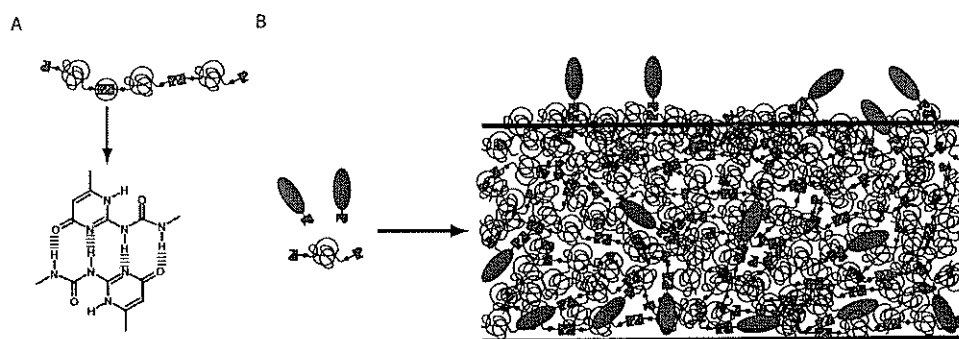
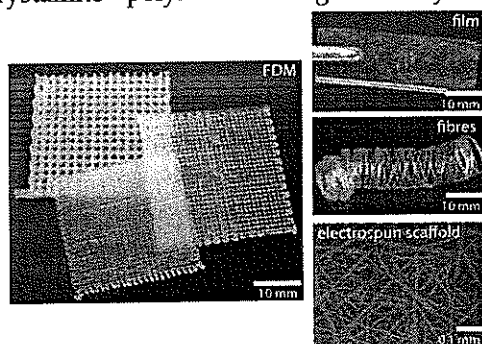


Figure 2. The modular approach to supramolecular biomaterials based on ureido-pyrimidinone (UPy) moieties. A. UPy-UPy dimer in a supramolecular polymer, B. the modular approach to bioactive supramolecular biomaterials.

Passive scaffolds

The feasibility of UPy-units in passive biomaterials was investigated in first instance. It was shown that supramolecular polymers consisting of FDA approved oligocaprolactones, end-functionalized with UPy-moieties, are eminently suitable as biomaterials. They can be easily processed into several scaffold morphologies varying from meshes, to films and grids, on which fibroblast cells were able to proliferate (fig. 3). Next to that, this UPy-polymer and other specially designed water-soluble UPy-moieties were shown to be biocompatible using several direct and indirect *in-vitro* toxicity studies. The oligocaprolactone UPy-polymer did not degrade *in vitro* during a period of more than 100 days, however, the degradation was accelerated when lipase enzymes were used. Comparable results were found for oligo(trimethylene carbonates) which changed from amorphous materials to semi-crystalline polymers owing to UPy-modification. Next to bifunctional UPy-polymers, also



trifunctional UPy-polymers were synthesized, resulting in different material properties. The UPy-modification of the oligo(trimethylene carbonates) allowed for easy processing at slightly elevated temperatures into stable 3D-scaffolds that did not flow below 50 °C because of the formation of supramolecular UPy-crosslinking.

Figure 3. Processability of the supramolecular UPy-materials into passive scaffolds.

Supramolecular nanorods. To tune the morphology and material properties of several UPy-modified oligocaprolactones the UPy-units were coupled to the prepolymers in three different manners: the UPy-group was directly attached (UPy), was coupled via a urethane functionality (UPy-urethane), or was attached via a urea group (UPy-urea). The directly coupled UPy-polymer showed only the formation of higher virtual molecular weight polymers by chain extension. The other two formed besides chain-extended polymers also UPy-UPy dimer stacks via urethane or urea hydrogen bonding in the lateral direction. Differences in material properties between the urethane and urea coupled UPy-polymers are caused by the length and dynamics of these nanorods owing to weaker versus stronger hydrogen bonding, respectively. This shows that binding motifs of UPy-units in combination with additional hydrogen bonds are important in determining the material morphology (fig. 4). In this way molecular structure can be coupled to the macroscopic properties of the materials.

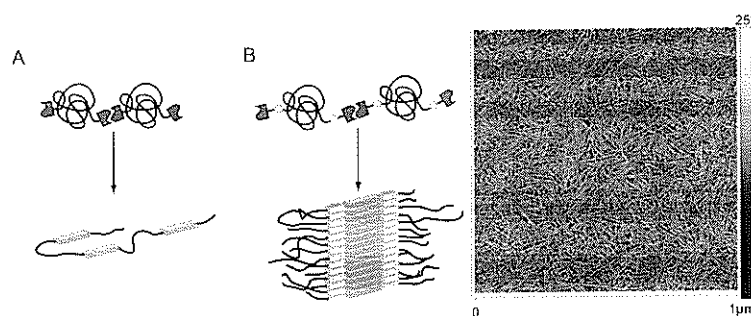


Figure 4. Schematic representations of the UPy-modified polymers. A. Direct UPy-functionalization and B. UPy-urea or UPy-urethane modification, which leads to the formation of nanorods (AFM measurement).

Co-polymeric biomaterials. Then, a modular approach was introduced to produce co-polymeric UPy-systems. First, co-polymeric systems were produced via intimate mixing of end-functionalized UPy-oligocaprolactone with UPy-modified poly(ethylene glycol) to regulate *in-vitro* degradability. Secondly, co-polymeric systems of bifunctional and chain-extended oligocaprolactones were made to tune the mechanical properties and tissue response *in vivo*. Surprisingly, a 20:80 mixture of both polymers with the chain-extended UPy-polymer in excess shows flexible properties without visible deformation upon implantation for 42 days (fig. 5). This mixture, a blend formed by intimate mixing through UPy-UPy interaction, shows a mild tissue response accompanied with the formation of a thin capsule. The material does not become more crystalline upon implantation. Hence, this mixture might be an ideal scaffold material for soft tissue engineering due to its flexibility and diminished fibrous tissue formation, and illustrates the strength of the modular approach.

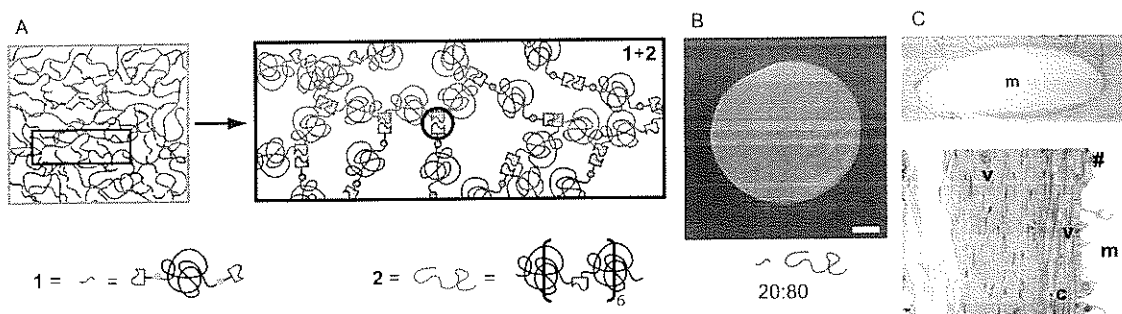


Figure 5. A. Intimate mixing of bifunctional (1) and chain-extended (2) UPy-polymers results in co-polymeric materials. B. The explanted disk of the 20:80 mixture and C. its histology after subcutaneous implantation.

Active scaffolds

A toolbox was designed containing different building blocks, varying from several UPy-polymers, to UPy-modified dyes, biotins, bioactive peptides and proteins. Several UPy-modified peptide sequences and two model proteins that can be used for the introduction of bioactivity into the UPy-modified polymeric materials were synthesized. A convenient solid-phase synthesis method was developed to functionalize peptide sequences with UPy-moieties on the solid support. UPy-peptides with an N-terminal cysteine were used to successfully functionalize green fluorescent protein mutants with UPy-moieties using native chemical ligation.

In order to prove the modular concept to bioactive biomaterials the UPy-functionalized oligocaprolactones were simply mixed with UPy-modified cell adhesion promoting GRGDS (Gly-Arg-Gly-Asp-Ser) and synergistic PHSRN (UPy-Pro-His-Ser-Arg-Asn) peptide sequences (fig. 6). The *in-vitro* results indicated strong and specific cell binding of fibroblasts to the UPy-functionalized bioactive materials containing both UPy-peptides. An even more striking effect was seen *in vivo* where the formation of single giant cells at the interface between bioactive material and tissue was triggered.

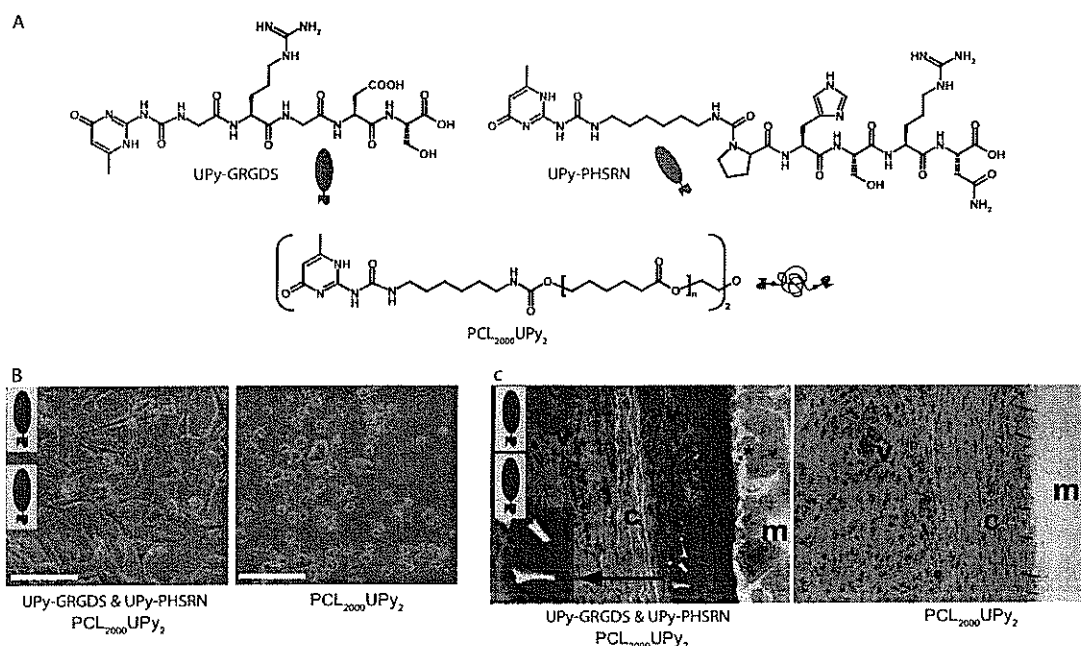


Figure 6. Bioactive supramolecular biomaterials have been produced consisting of A. the UPy-GRGDS and UPy-PHSRN peptides, and the UPy-modified oligocaprolactone. B. The behaviour of the active scaffolds is shown *in vitro* and C. *in vivo*.

Bioactive supramolecular nanorods. This biofunctionalization approach was taken a step further by introducing different binding motifs (i.e. UPy, UPy-urethane or UPy-urea) in both the UPy-peptides and UPy-polymers in order to regulate the stability and dynamics of the bioactive films by means of tuning of the UPy-UPy dimer nanorods. Extraction experiments of UPy-modified peptides out of UPy-polymers with these different motifs showed that all UPy-peptides (irrespective of the binding motif on the peptide) could be extracted better from the directly coupled UPy-polymer than from the UPy-urethane and UPy-urea polymers. This indicates that UPy-peptides might be better incorporated in the UPy-polymers with additional hydrogen bonds. Furthermore, UPy-peptides were more difficult to incorporate in the UPy-dimer urea nanorods than in the UPy-dimer urethane nanorods owing to the supposed higher association constant of UPy-urea stacks.

Besides that, several film preparation methods were investigated to tune ligand availability at the surface of the films showing that preparation procedures are very important in determining the final

properties of the biomaterial films. We propose that the binding of UPy-modified bioactives to UPy-polymers can be regulated by careful preparation of the films. The films have to be made via a supramolecular synthesis route applying the right amount of different solvents and in the right order. Films that were swollen with additional tetrahydrofuran showed a decreased presence and release of UPy-peptides and a diminished cell adhesion and tissue response. This indicates that bioactive surface properties are extremely sensitive. This sensitivity reflects again the dynamic behaviour of the supramolecular system.

Water-supramolecular polymer interface. Important is the stability of bioactive polymer films in an aqueous environment. We tried to investigate what happens at the water-supramolecular polymer interface. It has been shown that cells can adhere to cell adhesion UPy-peptides incorporated in the polymer films, indicating that the peptide-polymer interaction is strong enough to make this happen. Besides that, also *in-vivo* experiments showed the presence of UPy-modified cell adhesion peptides at the surface. It is assumed that the water-soluble UPy-peptides are partially incorporated in the UPy-UPy dimer stacks and that the polymers provide a hydrophobic shield around the UPy-bound peptide. These phenomena prevent total dissolution of the peptides in water. Similar results have been found when UPy-modified biotin was incorporated in UPy-polymer films, showing that avidin could bind to the biotin.

Epilogue

In conclusion, all experiments prove the modular concept of supramolecular (bioactive) biomaterials. To extend the approach, a dream scaffold might be produced in which different bioactive molecules are immobilized on different places in a composite 3D-material consisting of a combination of hydrogels and polymeric matrices which show different mechanical properties and degradation behaviour. In this way the scaffold might be able to regulate several cellular processes at the same time but also successively depending on the localization of the bioactive molecules.

Currently, we are applying the (bioactive) supramolecular biomaterials for tissue engineering of a bioartificial kidney. Using the concept and knowledge gained, versatile scaffold membranes are fabricated using ideal formulations of UPy-polymers in combination with UPy-biofunctionalities in order to trigger cells to proliferate, differentiate, stay viable and functional. Already some important issues in the development of these scaffold membranes for this bioartificial kidney have been tackled.