Corso di Dottorato di Ricerca

in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Biophysical characterization of nanostructures formed by self-assembled DNA derivatives for applications in biotechnology and biomedicine

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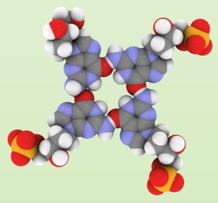
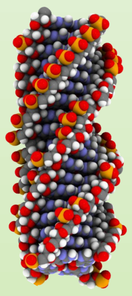
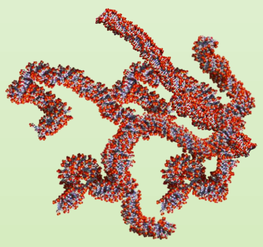


***G-quartet**, Guanosine (Gua) and guanosine 5'-monophosphate (GMP) a*



G-quadruplex



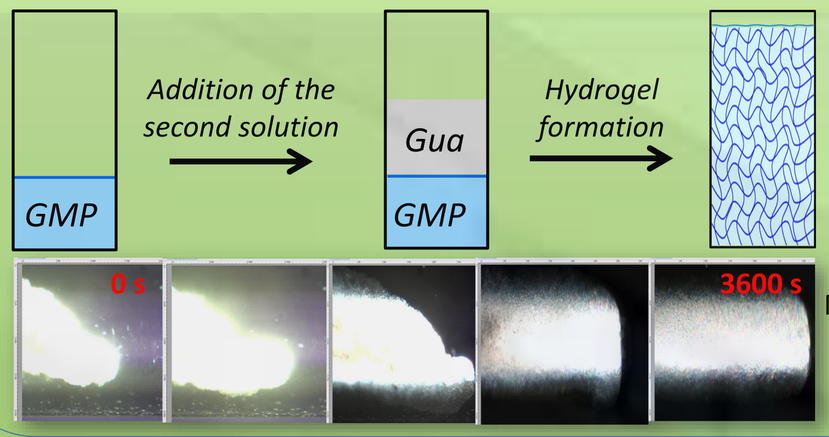
G-hydrogel

AIM OF THE STUDY

Guanosine 5'-monophosphate (**GMP**) and Guanosine (**Gua**) in water self-assemble in supramolecular, columnar helicoidal structures (**G-quadruplexes**), made by stacked planar tetramers (**G-quartets**) stabilized by non-covalent Hoogsteen bonds. Modulating G-quadruplex electrostatic repulsive and Van der Waals attractive forces by tuning the Gua/GMP molar ratio, supramolecular self-assembled **hydrogels** can be prepared. Stable G-hydrogels can be differentially hydrated modulating the amount of water from 80% and finally up to 98% v/v.cal

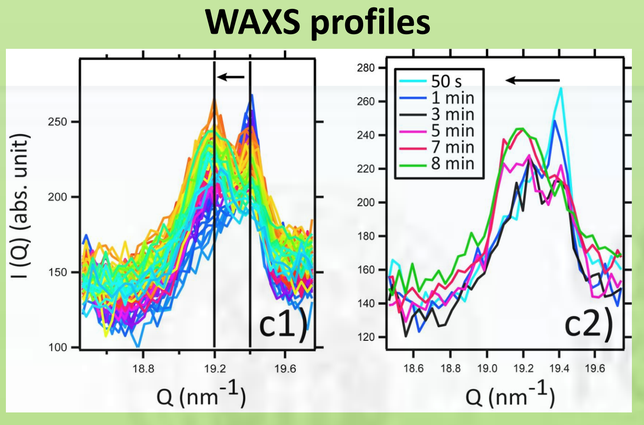
Here, a structural investigation of the **kinetic formation of the G-hydrogel** is reported, by using the time-resolved SAXS/WAXS at the Elettra SAXS beamline (Trieste, Italy). The SAXS results showed that the G-quadruplexs are formed in a few ms, while WAXS data gave us new results about the G-quartets stacking. It is interesting to note that the peak position shifts before stabilizing at the distance of 3.4 Å. Then, for the first time, we explored some biotech applications in which the G-hydrogel could be involved. Promising results were obtained in different fields. From the injected G-hydrogel we demonstrated that it is possible to get a **nanogel** (we characterized nanogel with a size around 150 nm) useful as a drug delivery system. G-hydrogel could be also a promising **bio-ink for 3D cell culture**. For this purpose, we performed cell viability assays, by using Human Dermal Fibroblastis cell line. Moving to an environmental application, we can claim that G-hydrogel is a promising system for **bioremediation**, in order to absorb heavy metals from wastewater and some surface.

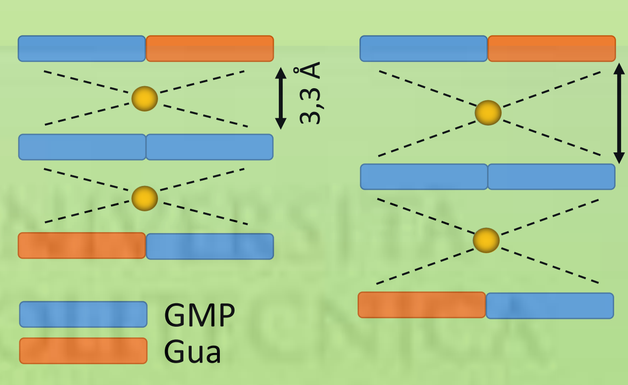
KINETIC FORMATION OF G-HYDROGEL



Microscopic evaluation of the Gua and GMP mixing in a capillary, followed by time

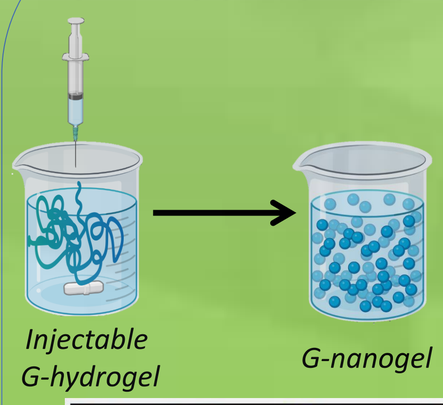
WAXS profiles





WAXS data highlights the formation of the G-quadruplexes due to the presence of the peak.
The time-resolved analysis shows the shift of the peak from a G-quartet distance of 3,3 Å to 3,4 Å.

G-NANOGEL: STRUCTURAL CHARACTERIZATION

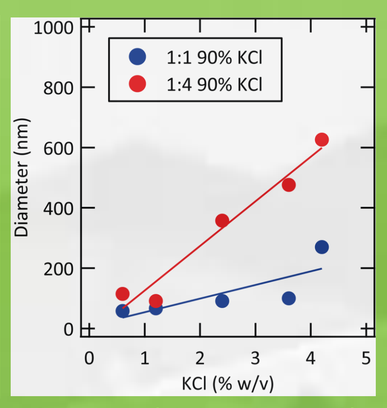


Injectable G-hydrogel → *G-nanogel*

- ✓ Charge stabilized by KCl and $CaCl_2$ salt at different concentrations (from 0,6 to 4,8 % w/v)
- ✓ G-nanogels stabilized in salt solution by Poloxamer 408 at 0,6% w/v.
- ✓ Size nanoparticles remain stable until one month (~ 150 nm).
- ✓ DLS and AFM techniques confirm the presence of G-nanogels and their size.
- ✓ Confocal microscopy highlights the possibility to load the nanogel with a fluorescence molecule (i.e. THT).

G-nanogel + 3,6 % KCl
AFM

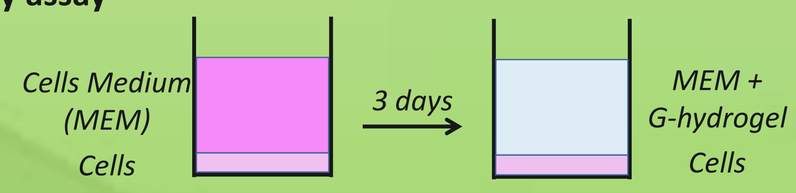
G-nanogel + 3,6 % KCl + THT
Confocal Microscopy



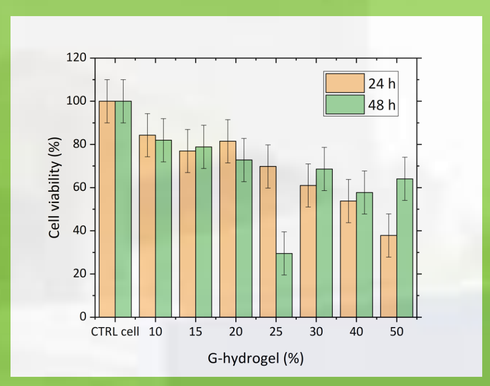
G-nanogel 1:1 vs 1:4 + KCl
DLS

G-HYDROGEL AS A BIO-INK FOR 3D CELL CULTURE (NHDF cell line)

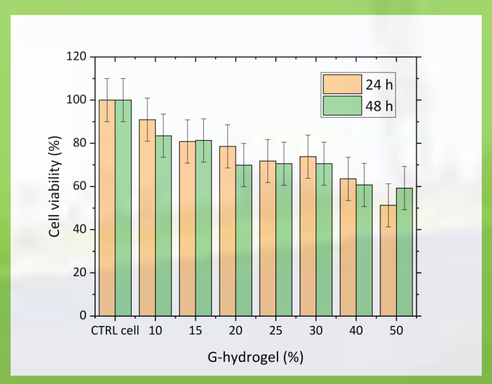
Cell viability assay



For the first time, we evaluated the viability of NHDF cells grown on the bottom of a well plate. After 3 days the cell medium was changed with different concentrations of G-hydrogel (from 10 to 50 %).

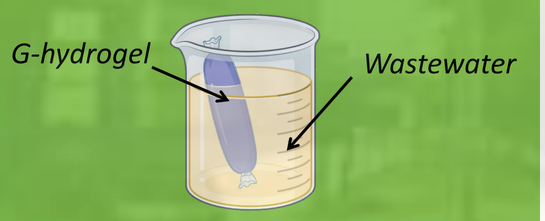


1:2 97% G-hydrogel
pH_{gel} = 8,5 - 9

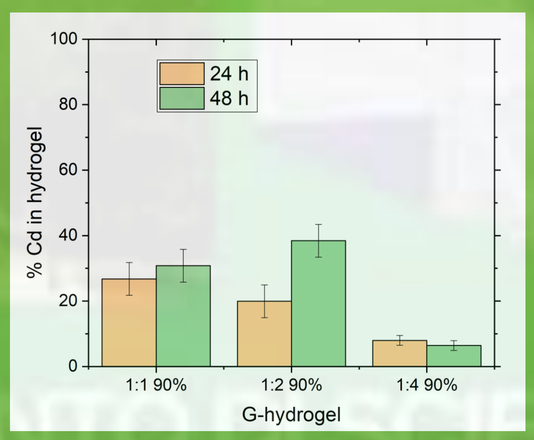


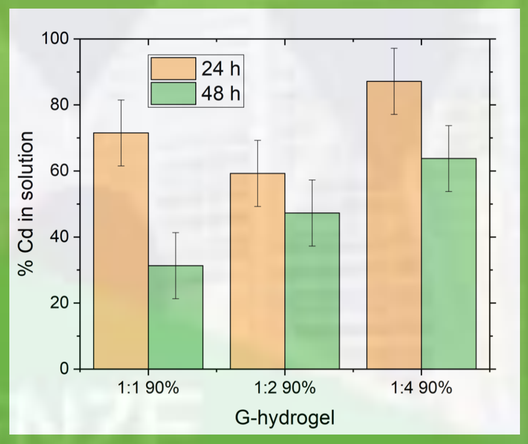
1:2 97% G-hydrogel
pH_{gel} = 7,6

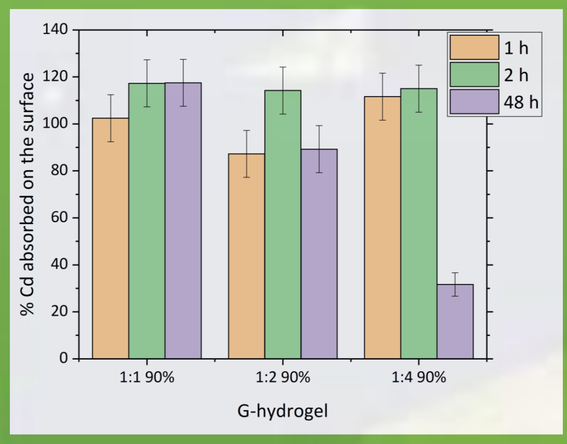
G-HYDROGEL FOR THE BIOREMEDIATION OF WASTEWATER AND SURFACE



Molecules absorption from G-hydrogel







Cadmium is the heavy metal used to test the absorption capacity of G-hydrogel, both when it is dissolved in a solution (i.e. wastewater) and when it is on a surface (i.e. glass). Experiments were performed for different hydrogel concentration and followed by time.

REFERENCES

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4. Bai Qu et al., Int. J. Mol. Sci., 152 (2020), 437-448