An injectable *in situ* forming composite gel to guide bone regeneration: design and development of technology platform

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INTRODUCTION: Various clinical situations such as augmentation of osteoporotic fractures, treatment of bone defects and specific indications in spine surgery can benefit of injectable composite gels with self-setting features [1]. The aims of this research project are i) to develop a novel injectable/mouldable bone graft substitute which consists of an approved bone graft substitute of natural origin with excellent biofunctionality suspended in a thermosensitive, biocompatible and biodegradable polymer solution and ii) to assess the feasibility of using the *in situ* forming composite gel (ISFcG) as filling or grafting material suitable to support tissue regeneration in dental/orthopaedic surgery.

METHODS: Several formulations of thermogelling polymer solution based on chitosan and glycerol phosphate disodium salt were prepared and tested as delivery vehicle for Orthoss® spongious granules (Geistlich Pharma AG, Wolhusen, Switzerland) to obtain the final ISFcG. All basic components of the polymer solution are of natural origin, biodegradable, biocompatible and already approved by regulatory agencies for human use. The final formulation was selected by determination of i) swelling and resistance, ii) mechanical and chemical stability, iii) cell seeding capacity and long-term cell growth and iv) long-term stability (6 months) of various polymer compositions.

RESULTS: All ISFcGs were rapidly (30 sec) and completely re-hydrated when KRH buffer was added. Samples were easy to handle by spatula with good adaptability and robustness (**Figure 1**). They successfully maintained the morphological and physical integrity during the mechanical and chemical stability studies, and they effectively entrapped the Orthoss® granules into the polymer network.

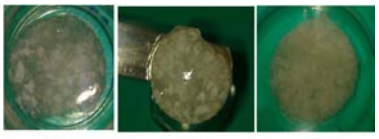

Figure 1 Re-hydration performances of ISFcG: (a) after 30 sec, (c) 6 h and (d) 8 days.

Good cell seeding capacity was observed for all ISFcGs, ranging between 30-40%. Good cell proliferation was shown after 28 days in culture (**Figure 2**), demonstrating that the excellent bioconductive properties of Orthoss® granules were preserved when they were incorporated into chitosan polymer matrix.

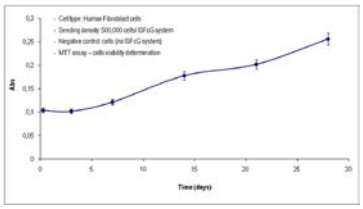

Figure 2 Proliferation of adult fibroblasts in ISFcG over a period of 28 days, at 37°C, 5% CO_2 in DMEM supplemented with 10% FBS.

The final ISFcG presented optimal stability either in simulated media (KRH) or whole human blood for 8 days. Bone graft granules were entrapped and maintained within the polymer matrix for more than 8 days, no evidence of gel disaggregation was visible (**Figure 3**).

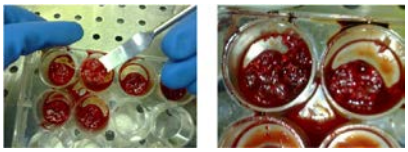

Figure 3 ISFcG embedded into whole human blood after 8 days.

The long-term stability (6 months) of final ISFcGs performed at +5±2°C did not reveal any evidence of physical instability such as release of spongious granules or uncontrolled discharge of polymer matrix. The final ISFcG was developed both as a ready to use injectable formulation and in the freeze-dried form (solid form) which can be easily and promptly re-hydrated to a mouldable consistency.

DISCUSSION & CONCLUSIONS: The excellent stability and the good capacity to support cell growth in addition to its injectable/mouldable features make this system a promising candidate as scaffold for bone regeneration..

REFERENCES: [1]Sune Larsson, et al (2011) *Injury, Int. J Care Injured 42 S30-S34.*