Self-organising clay gels for preventing bone infections in orthopaedic graft/implant therapies

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Introduction

Infection is a leading proponent of clinical orthopaedic implant failure. In addition, implant loosening due to a lack of osteogenic integration between the tissue and implant results in revision orthopaedic implant surgery. Enhancing the implant surface properties to initiate bone growth while simultaneously preventing microbial adhesion will be critical for clinical efficacy of orthopaedic implant surgery.

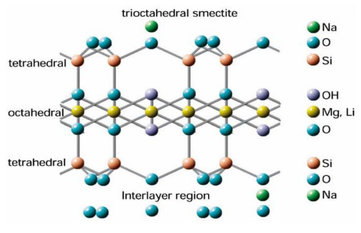


Fig 1. The 2:1 layered structure of Laponite consisting of two tetrahedral silica sheets sandwiching a central octahedral magnesia sheet

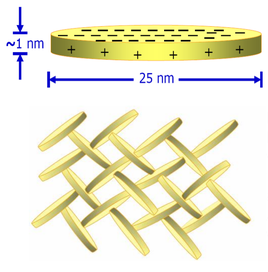


Fig 2. When dispersed in water Laponite particles self-organise via electrostatic interaction to form thixotropic gels. Figs 1&2 adapted from Rockwood Additives Ltd., 2005, www.laponite.com

3-D matrices serve to deliver and maintain, at the site of damage, the requisite extracellular micro-environment for stem cell-mediated tissue regeneration. Certain clays are well-known for their ability both to self-organise into colloidal gel/film networks *and* to adsorb biological molecules, owing to the large and highly charged specific surface area of the nano/micro-sized clay particles. Laponite is a synthetic clay with wide use in cosmetics. When dispersed in distilled water, the nano-sized Laponite particles self-organise over time to form a gel network (Figs 1-2).

We are exploring the potential utility of clay-gels for bone graft/implant integration[1]. Here we present proof-of concept for the potential of this approach for enhanced osteogenesis and reduced infection in orthopaedic implant bone repair.

Aim

To assess the potential of clay-based gels for osteogenic and antimicrobial resistant localization for graft/implant skeletal repair.

I. Clay gel films localise proteins and enhance the alkaline phosphatase response of C2C12 cells to BMP2.

Methods

Laponite XRD powder (Rockwood Additives Ltd, UK) was added to distilled water under rapid agitation. Laponite concentrations of 2.5% used throughout unless stated **Laponite films:** Tissue culture plastic surfaces and decellularised samples of human trabecular bone surfaces were rinsed with saline and air-dried. Minimal volumes of 2% wt/vol laponite suspensions were applied to surfaces and air-dried. C2C12 cells were seeded onto surfaces with or without BMP2 (100ng/ml). After 3 days samples were stained for alkaline phosphatase activity.

Results

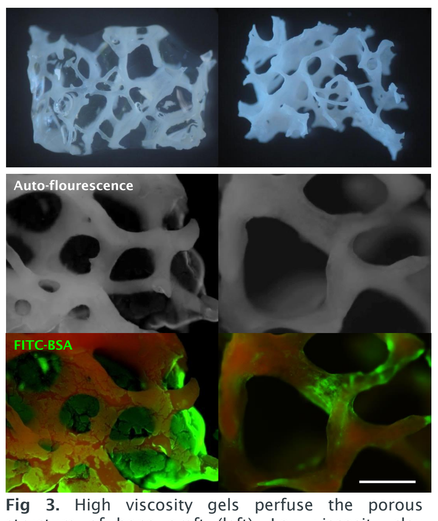


Fig 3. High viscosity gels perfuse the porous structure of bone graft (left). Low viscosity clay suspensions coat the bone matrix surface (right). Both approaches allow for the localisation of proteins (FITC-BSA, bottom panels). Scale = 200 μm.

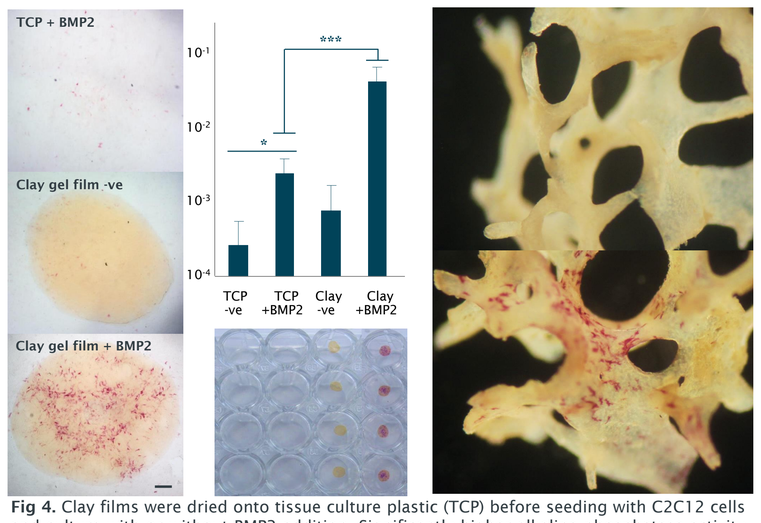


Fig 4. Clay films were dried onto tissue culture plastic (TCP) before seeding with C2C12 cells and culture with or without BMP2 addition. Significantly higher alkaline phosphatase activity in response to BMP2 was observed in cells cultured upon clay films (left and middle; *y* axis = absorbance). Using the same approach, clay films were applied to bone graft and the response to BMP2 was enhanced in the presence of clay (right). Scale = 250 μm.

[1] Dawson JI, Kanczler JM, Yang XB, Attard GS, Oreffo ROC. Clay Gels For the Delivery of Regenerative Microenvironments. *Advanced Materials*. 2011; 23: 3304-3308; Dawson JI, Oreffo, ROC. Clay: New opportunities for tissue regeneration and biomaterial design. *Advanced Materials* 2013; 25: 4069-86

II. Clay (Laponite-chlorhexidine) hydrogel / films inhibition of microbial attachment and growth *in vitro.*

Methods

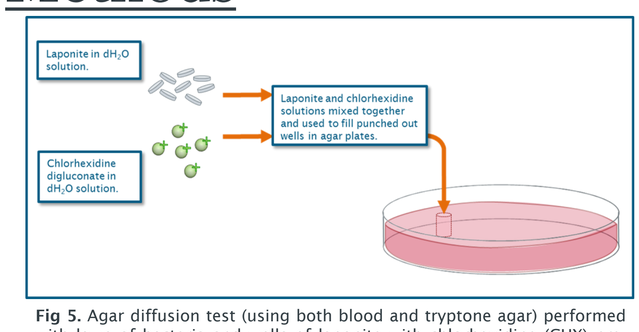


Fig 5. Agar diffusion test (using both blood and tryptone agar) performed with lawn of bacteria and wells of laponite with chlorhexidine (CHX) pre-mixed in. Plates incubated overnight.

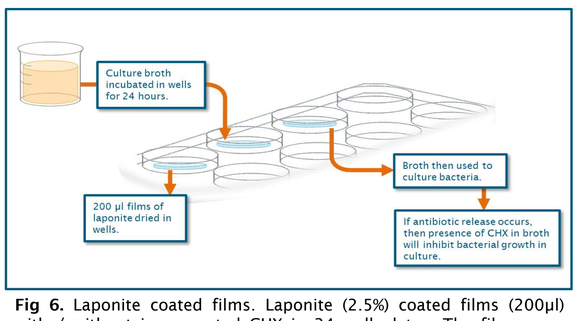
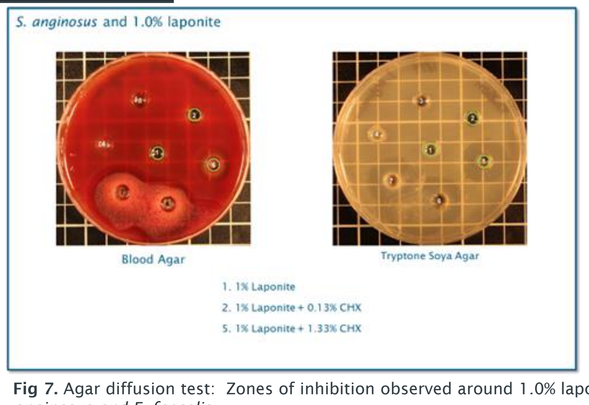


Fig 6. Laponite coated films. Laponite (2.5%) coated films (200µl) with / without incorporated CHX in 24 well plates. The films were incubated with culture broth (either 500 µl or 1000 µl brain heart infusion broth) for 24hrs before addition of the bacteria cultures10^2 CFU/ml). (n=4). Bacteria growth was measured by optical density (absorbance at 600nm).

Results



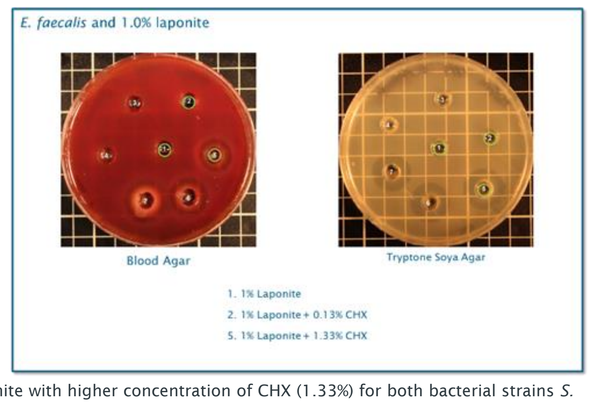


Fig 7. Agar diffusion test: Zones of inhibition observed around 1.0% laponite with higher concentration of CHX (1.33%) for both bacterial strains *S. anginosus and E. faecalis*.

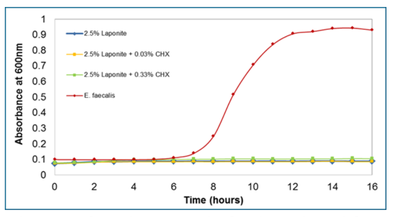


Fig. 8. *E. faecalis* - 200 µl of gel incubated for 24 hours with 500 µl of brain heart infusion broth. Broth used to grow 10^2 CFU/ml of bacteria

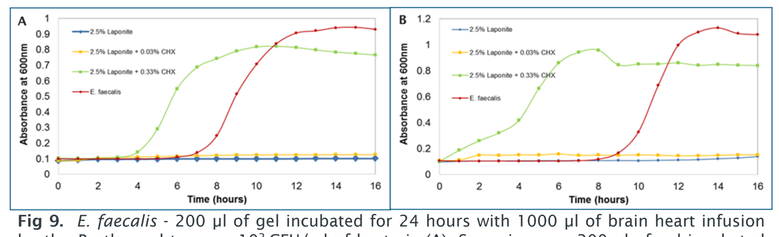


Fig 9. *E. faecalis* - 200 µl of gel incubated for 24 hours with 1000 µl of brain heart infusion broth. Broth used to grow 10^2 CFU/ml of bacteria (A). S. anginosus - 200 µl of gel incubated for 24 hours with 1000 µl of brain heart infusion broth. Broth used to grow 10^2 CFU/ml of bacteria (B).

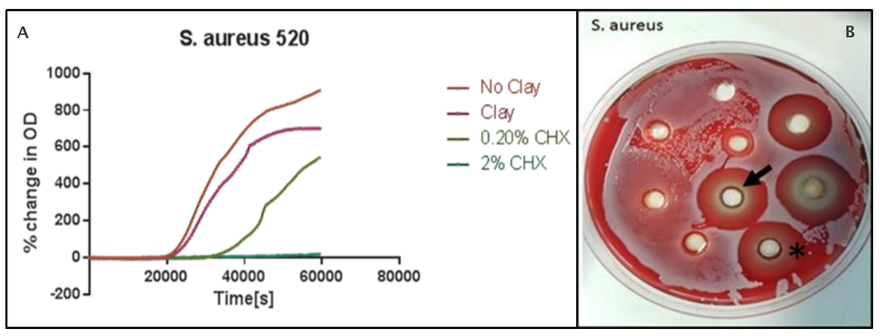


Fig 10. Reduction in *S. Aureus* growth with addition of CHX with clay (A), Image of the culture plate demonstrating inhibition of bacterial growth around the clay gel and CHX (B arrow indicates 1.0% Laponite with 2.0% CHX; * indicates 1.9% Laponite 0.2% CHX).

Discussion

These initial studies show the feasibility of using Laponite hydrogels as a vehicle to carry osteogenic factors to promote cellular skeletal differentiation, and to carry a localised antimicrobial agent with its functionality maintained during uptake to the gel and release into the surrounding culture media.

The antimicrobial mechanism of the agent used within these tests, chlorhexidine, remained unaltered. Functionality however is dependent upon the concentrations used in formulating the nanoclay-antimicrobial gel and may need adjustment for specific strains of bacteria. Therefore, it will be beneficial to conduct further work looking into concentration refinement to develop a gel that is effective against a broad spectrum of bacteria and also to provide initial release of unbound antimicrobials into surrounding areas of tissue if required. In addition, the potential efficacy of these thixotropic clay films with dual osteogenic and antimicrobial adhesion agents will serve as a critical step in the prevention of orthopaedic implant failure due to lack of osseo-integration and/or pre operative and post operative contaminations.

Acknowledgements

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