The Potential Functions of Hyaluronic Acid as an Extra-Scaffold and a Carrier for Growth Factors in the Bone Healing Process

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Background

Cells, growth factors (GFs), and scaffold are three essential factors for tissue engineering.

GFs improve angiogenesis, cell proliferation, and extracellular matrix production with continuous controlled exposure in an active form. Our previous studies suggested that the multiple applications of human amnion growth factors (AGF) into osseous defect can "mimic in-utero" growth. However, multiple applications is challenging in clinical use. To help decrease the number of applications needed, an additional carrier or scaffold would beneficial.

Osteoconductive materials are also essential to serve as a scaffold onto which bone cells (osteoblasts and osteoclasts) can attach, migrate, proliferate and differentiate. However, there is a micro-gap between scaffold and recipient site that affects the cellular migration, which is why an accessory or extra scaffold can help bridge these micro-gaps.

Hyaluronic acid (HA) is a high molecular weighted linear polysaccharide with disaccharide repeats of D-glucuronic and N-acetyl-D-glucosamine. It is distributed widely throughout the extracellular matrix, connective tissue, the epithelial tissue, the neural tissues, and body fluids. Even though the HA structure is a simple polysaccharide, it has an array of biological functions such as: lubrication for joints, wound healing, preserving bone strength, among many others. According to previous studies, it has been suggested that HA has the capability to hold water; therefore, this experimental design was created to implement similar concept with GFs. Upon further review, some studies have proposed that HA may have the ability to hold certain types of GFs and slowly release these them in an active form.

We hypothesized that HA could improve bone healing as a carrier and gradually release the active form of AGF hence contributing as an extra scaffold that could cover the micro-gaps and enhance the cellular migration thus improving the clinical outcome.

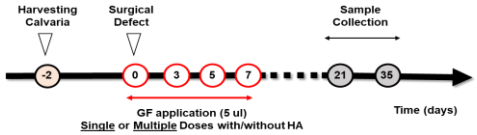
Materials and Methods

I. Experimental Groups (each n=5)

Group 1: Scaffold with a Single Dose of AGF (1x AGF)
- **1A: without HA**
- **1B: with HA (0.0625%)**
- **1C: with HA (0.125%)**
- **1D: with HA (0.25%)**

Group 2: Scaffold with Multiple Doses AGFs (4x AGF)
- **2A: without HA**
- **2B: with HA (0.0625%)**
- **2C: with HA (0.125%)**
- **2D: with HA (0.25%)**

II. Timeline



III. Experimental Design

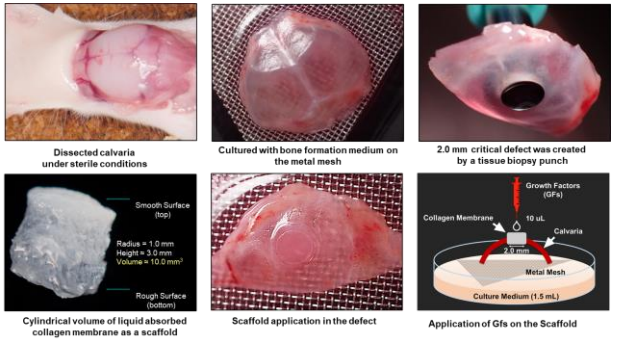


Figure 1: Surgical Procedure
50 Calvaria from 7-9 days old CD1 neonatal mice were harvested under sterile conditions. A 2 mm defect on the calvaria, created by tissue biopsy punch and type I collagen membrane as a scaffold and applied AGF with or without different HA concentrations. Calvaria were then cultured for 35 days. The culture medium was changed every 2-3 days and collected for Alkaline Phosphatase (ALP) activities.

Results

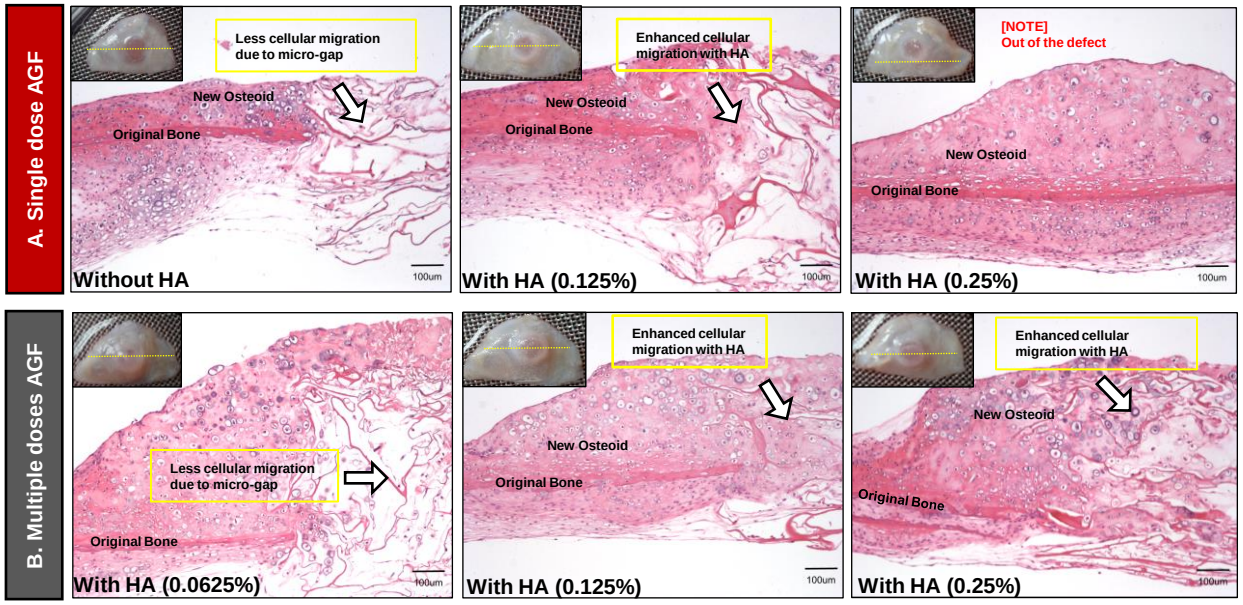


Figure 2: Histological Analysis in a Dose-Dependent Manner of HA

A: Single Dose AGF with or without HA
A single dose of AGF with HA can improve bone formation sanctioning a seamless surface healing around the defect area. Single dose of AGF without HA shows some cellular migration into the scaffold area, but it could not infiltrate into the micro-gaps. 0.125% HA with a single dose of AGF shows more cellular infiltration into the defect area. It appears to fill the micro-gaps.

B: Multiple Doses AGF with HA.
0.0625% HA with multiple doses of AGF shows some cellular migration into the scaffold area but does not entirely fill in the micro-gaps as seen in single dose of AGF with 0.125% HA. This suggests that a higher concentration of HA is would be better in combination with AGF. With 0.125% HA with 4x AGF, there is a lot more cellular infiltration and bridging of the micro-gaps, which was not seen with 0.0625%. To find the optimal dose of HA needed to cover these micro-gaps 0.25% HA was used with 4x AGF. This shows far more cellular infiltration and bridging of the micro-gaps suggesting that a higher concentration of HA would be beneficial with AGF.

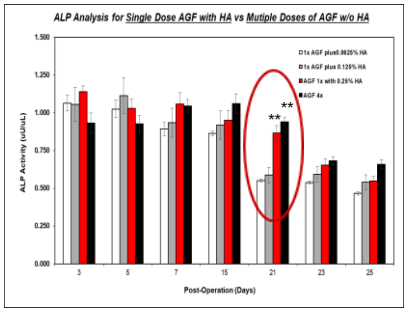


Figure 3: ALP Analysis

The ALP activity of 1x AGF with 0.0625% HA and 0.125% HA groups reduced gradually in a time-dependent manner. However, the activity of 1x AGF with 0.25% HA and 4x AGF without HA groups remained until day 21 in comparison.

** indicates statistically significant ($p < 0.01$), one-way ANOVA with post-hoc Turkey HSD test. Mean +/-SEM

Conclusion: Previous results indicate that multiple applications of AGF achieved better bone formation in comparison to single dose of AGF alone. However, with the use of HA as an extra scaffold and a carrier, a single dose of AGF can achieve similar bone formation in comparison to multiple dosage of AGF and thereby reduce the number of clinical application needed which improves the clinical outcome.