GrowDex®
– NATURAL GROWTH –

Automation of 3D cell culture using cellulose-based scaffolds

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Project goal

3D cell culture systems are emerging and show potential for better simulating the *in vivo* tumor microenvironment and for eliminating species differences allowing drug testing directly in human-based systems before drugs move into clinical trials.

The purpose of this study was to automate the production and cultivation of the human primary osteogenic sarcoma cell line, SaOS-2, in:
➢ a scaffold-based system, multiple spheroids in GrowDex® (GDS, Fig.1A and B)
➢ a scaffold-free system, single spheroids (SS, Fig.1C), for high-throughput screening

Furthermore, the effect of GrowDex to mimic the physiological tissue stiffness compared to a scaffold-free culture system was examined.

Key Findings

✓ Automation compatibility of viscous hydrogel such as GrowDex
✓ Successful scripts establishment for the production and maintenance of GrowDex multiple spheroids and scaffold-free models
✓ GrowDex high potential for high-throughput drug screening and model stiffness adjustments to better simulate *in vivo* conditions
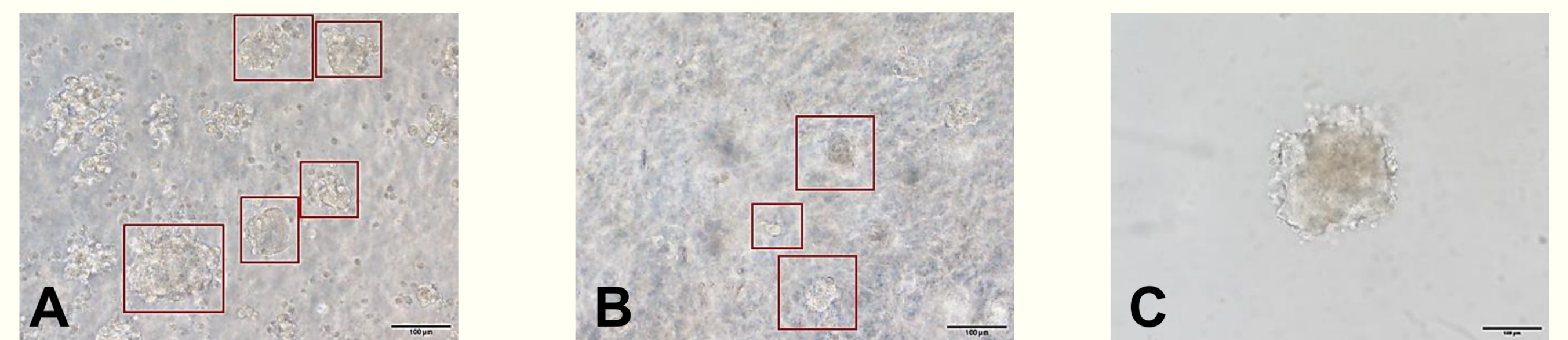

Figure 1. Microscopy pictures of SaOS-2 spheroids in 0.5% GrowDex at different Z positions (GDS, **A** and **B**) and in ULA round bottom well plates (SS, **C**) at day 11. Scale bar, 100µm

Project data

– Experiments were conducted on the Fluent® 780 automation workstation (Fig.2A). The scripts for automated model production and media exchange were established using the FluentControl™. Technical parameters such as aspiration and dispensing speed as well as XYZ dispensing positions were empirically defined.

– The established scripts allowed the production of multiple spheroids in GrowDex as well as the aggregation of SaOS-2 in single spheroids in the U-bottom ULA plates (Fig.1). Automated media exchange was successfully performed without altering the shape and position of the spheroids in the wells.

– GDS and SS remained stable for 11 days and increased in size over time, showing a similar growth rate (Fig.2B). Viable GDS populated the entire sample at different Z-positions with a compact morphology (Fig.3A and B), a parameter that characterized the SS too (Fig.3C).
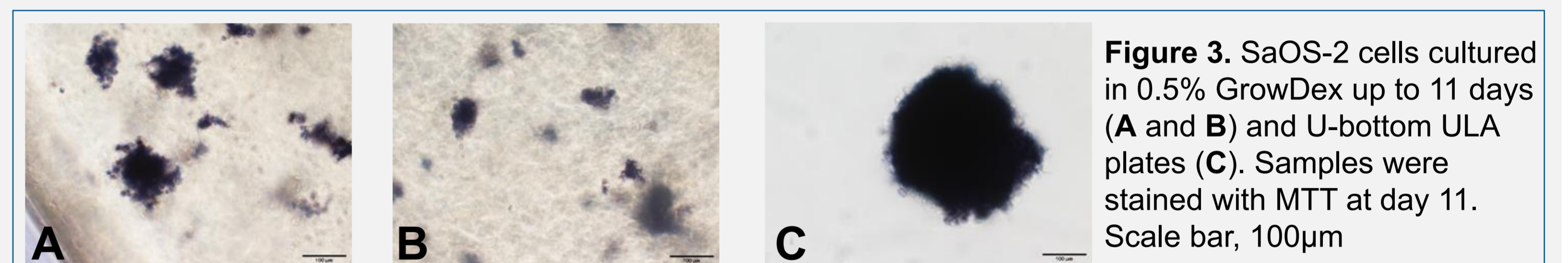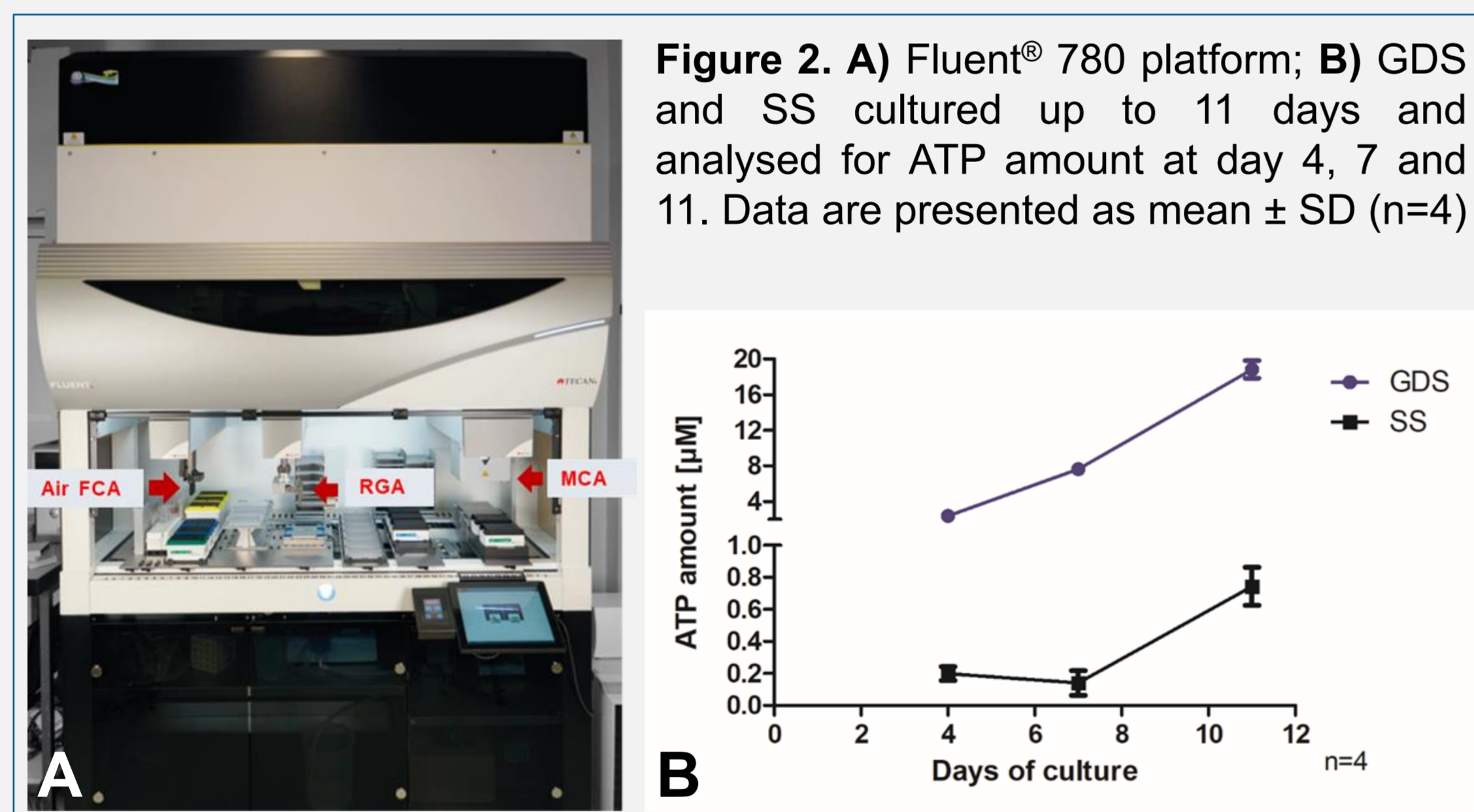

Figure 2. A) Fluent® 780 platform; **B)** GDS and SS cultured up to 11 days and analysed for ATP amount at day 4, 7 and 11. Data are presented as mean ± SD (n=4)

Figure 3. SaOS-2 cells cultured in 0.5% GrowDex up to 11 days (**A** and **B**) and U-bottom ULA plates (**C**). Samples were stained with MTT at day 11. Scale bar, 100µm
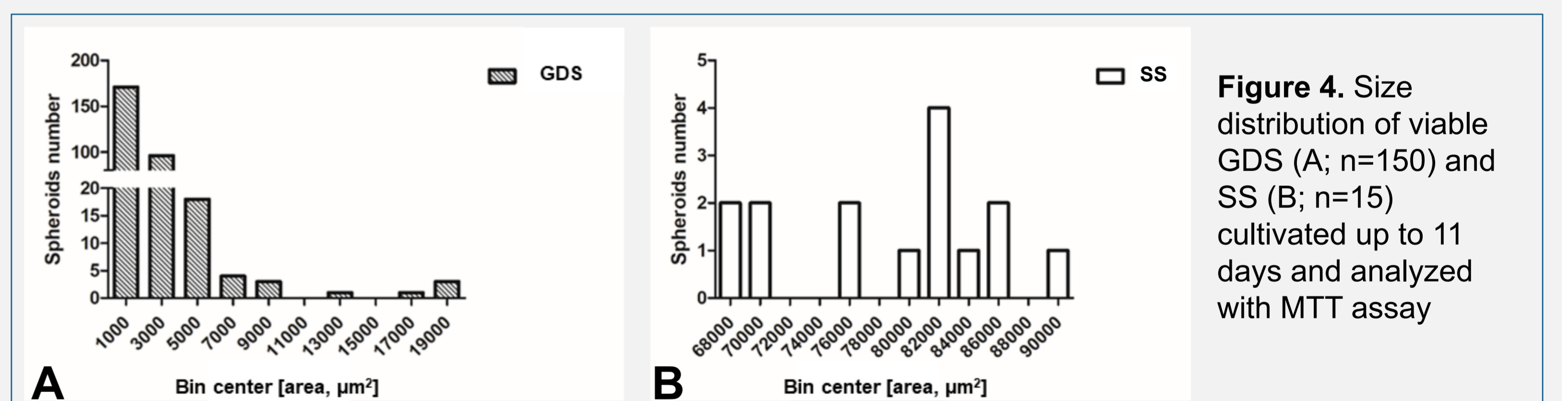

Figure 4. Size distribution of viable GDS (A; n=150) and SS (B; n=15) cultivated up to 11 days and analyzed with MTT assay

– GDS showed a wide size distribution; while SS were bigger and more homogenous in size in comparison (SS area was 10-fold larger than GDS area) (Fig.4).

– SaOS-2 responded in both systems to taxol (Fig.5A) and doxorubicin (Fig.5B), showing higher IC_{50}-values for GDS compared to SS. Taxol and doxorubicin were 3.5- and 4.5-fold respectively, more potent in SS than in GDS.

– At high drug concentrations, SS were characterized by collapsed morphology with a viable, compact core and a loose outer cell layer (Fig.6).
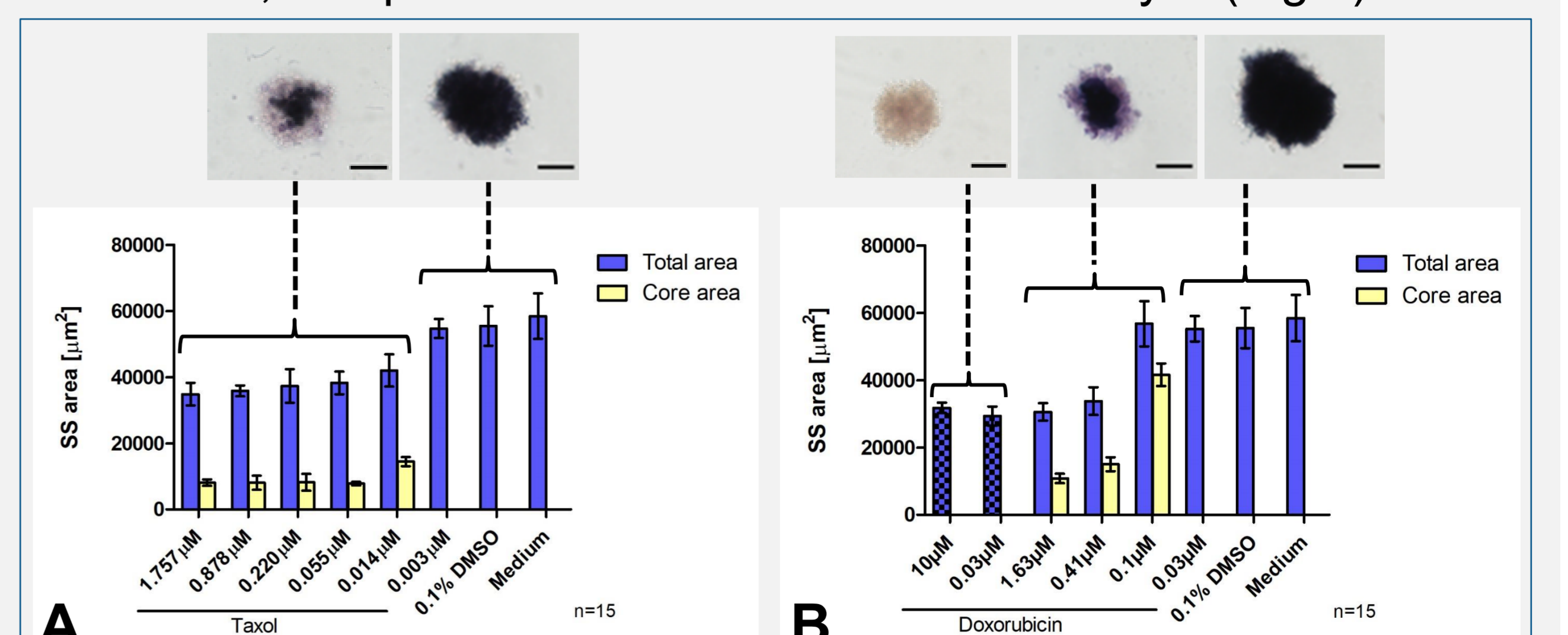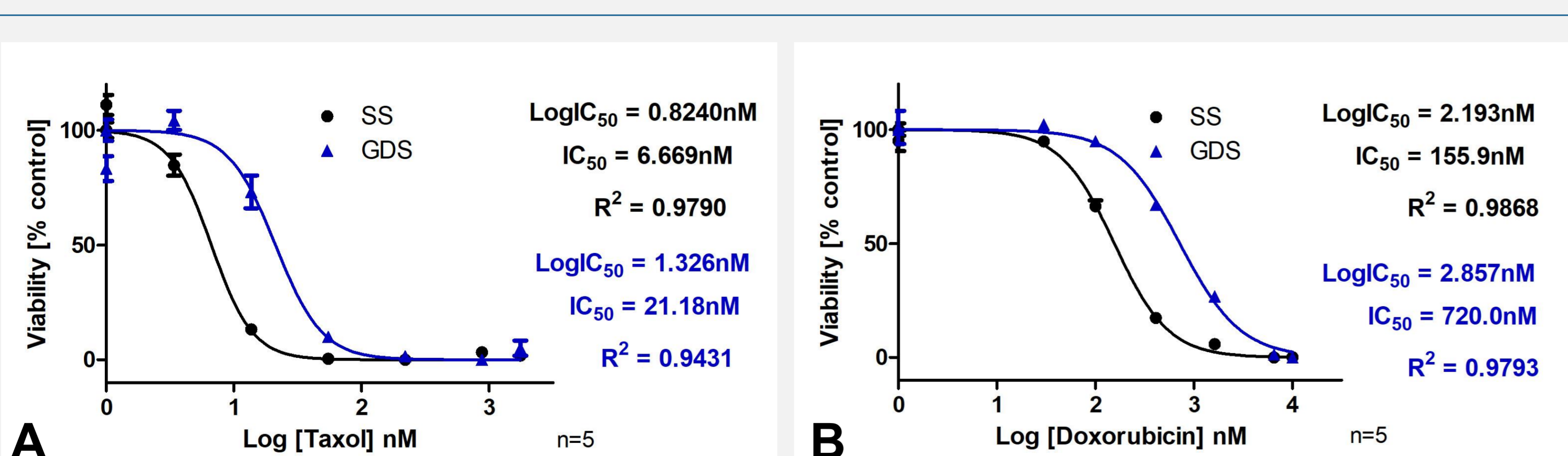
– No morphology change in GDS.

Figure 5. Taxol (**A**) and doxorubicin (**B**) effect on GDS and SS. Viability was determined at day 7 (72h drug treatment) quantifying the amount of ATP. Data are shown as mean ± SEM (n=5)

Figure 6. Area size of SS treated with taxol (**A**) and doxorubicin (**B**). Data are shown as mean ± SD (n=15)

Conclusions

✓ Automation protocols could be established with high reproducibility for the generation and maintenance of scaffold-based and scaffold-free models
✓ GrowDex hydrogel is automation-compatible
✓ Successful generation of automated dose-response experiments
✓ GDS are less sensible to drugs than SS